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BREEDING BEHAVIOUR PATTERNS AND ECOLOGICAL DIFFERENCES BETWEEN *TILAPIA* SPECIES AND THEIR SIGNIFICANCE FOR EVOLUTION WITHIN THE GENUS *TILAPIA* (PISCES : CICHLIDAE)

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[Communicated by Dr L. Harrison Matthews, F.R.S.—Accepted 15th April 1958]

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I. SOURCES OF INFORMATION

In recent years cichlid fish have been much used for experimental work in aquaria on various aspects of breeding behaviour, but as yet there is little published information relating differences in behaviour to ecological differences between species. The aim of this paper is to collate data from field studies

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on many species of *Tilapia* in the waters of East and Central Africa, to relate the types of breeding behaviour to the ecological background, and to try to assess the evolutionary significance of the patterns of breeding behaviour and ecological differences which emerge. Behaviour and ecological studies present complementary pictures of the interaction between the fish and its environment, and an attempt is made here to piece these together to examine the evolutionary effects of their reciprocal and complex interaction.

The data considered in this paper are drawn from my own observations made in natural waters in East and Central Africa from 1945 to 1956 on the following species of *Tilapia* :

Lake Nyasa species, *T. squamipinnis* Günther, *T. lidole* Trewavas, 1941, *T. karongae* Trewavas, 1941, *T. saka* Lowe, 1953, *T. shirana* Boulenger, and *T. melanopleura* Dumeril ; L. Victoria species, *T. variabilis* Boulenger and *T. esculenta* Graham, 1929 ; *T. nilotica* Linné from many East African lakes together with *T. leucosticta* Trewavas, 1933, *T. galilaea* Linné and *T. zillii* Gervais in Western Uganda ; Tanganyika species, *T. karomo* Poll, 1948 and *T. tanganicae* (Günther) ; the eastward-flowing river system species, *T. pangani* Lowe 1955, *T. girigan* Lowe, 1955 and *T. jipe* Lowe, 1955, together with some observations on *T. mossambica* Peters, *T. hunteri* Günther and *T. nigra* Günther ; Central African species, *T. sparrmani* A. Smith, *T. macrochir* Boulenger and *T. andersonii* Castelneau.

Detailed observations on some aspects of the biology of these species have been published elsewhere (Lowe, 1952, 1953, 1955 a, b and c, 1956 a and b, 1957 and 1958). Identifications were checked at the British Museum Natural History with the kind assistance of Dr E. Trewavas.

Tilapia have been studied extensively as they are of great economic importance as food fish, and additional information on the biology of various species has been culled from the following sources :

Lake Nyasa species, Bertram, Borley & Trewavas, 1942 ; Trewavas, 1941 ;
Lake Victoria species, Graham 1928 and 1929 ; Fish, 1951 ; Greenwood, 1953 ;
T. nilotica and associated species, Boulenger, 1908 ; Capart, 1955 ; Daget, 1954 ; Fish, 1955 ; Liebman, 1933 ; Poll, 1932, 1939 a and b ; Trewavas, 1933 ; Worthington, 1929, 1932 ; Worthington & Ricardo, 1936 ; and Annual Reports of the Game and Fisheries Department Uganda Protectorate ;
Tanganyika species, Poll, 1946, 1948, 1956 ;
Belgian Congo species (mainly *T. macrochir* and *T. melanopleura*), de Bont, 1950 ; de Bont, Halain, Huet & Hulot, 1948 ;
T. melanopleura and other species in West Africa, Daget, 1954 ; Svensson, 1933 ; Irvine, 1947 ; Welman, 1948 ;
T. zillii, Daget, 1952 ; El Zarka, 1956 ;
South African and Rhodesian species (mainly *T. mossambica*, *T. melanopleura* and *T. sparrmani*), Barnard, 1949 ; Hey, 1952 ; Jubb, 1952 ; du Plessis, 1946 ; du Plessis & Groenewald, 1953 ; Ricardo, 1939 a ; also reports of the Northern Rhodesia Game, Tsetse Control & Fisheries Department ; *T. mossambica*, Vaas & Hofstede, 1952 ;
T. nigra, Brown & van Someren, 1953 ; Annual Reports of the Kenya Game & Fisheries Department ;

T. rukwaensis Hilgendorf & Pappenheim, Ricardo, 1939 a and b ; Swynnerton (unpublished report).

Among the most intensive studies of *Tilapia* breeding behaviour in aquaria so far published are those carried out in America by Aronson (1945, 1949, 1951, 1957) on the West African species *T. macrocephala* (Bleeker) (synonymous with *T. heudeloti* Dumeril, vide Trewavas, 1947 a), and in Europe by Baerends and Baerends-van Roon (1950) on *T. natalensis* M. Weber (synonymous with *T. mossambica* Peters, vide Trewavas, 1937). Seitz (1948) has also made intensive studies on " *T. heudeloti* " and " *T. natalensis* " but there appears to be some doubt about the identification of the species used in his experiments (Aronson, 1949), and Trewavas (personal communication) has reason to believe that they were both forms of *T. mossambica* Peters. Many species of *Tilapia* are morphologically very alike, and there has been considerable confusion over the naming of material used in experimental work.

T. macrocephala studied by Aronson is a mouth-brooder in which the male normally broods the eggs and young in his mouth, hereafter referred to as a " paternal-brooder ". Of the twenty-five *Tilapia* species studied personally and by the above listed authors *T. sparrmani*, *T. melanopleura* and *T. zillii* are " substratum-spawners " in which the eggs and young are guarded by both parents but not brooded in the mouth. In all the other species except *T. macrocephala* the female normally broods the eggs and young in her mouth (" maternal-brooders "). Only one other species, *T. guinasana* Trewavas, 1936, from South-west Africa and apparently very closely related to *T. sparrmani*, is known from aquarium studies to be a substratum-spawner (Aronson, 1949 quoting Roloff, 1938 & 1939). *T. macrocephala* stands out as an exception in showing the paternal-brooding pattern of behaviour, and it is unfortunate that generalisations have been made from the behaviour of this species which is in many ways different from that of the majority of *Tilapia*. While it is likely that further investigations, particularly in West Africa and the Congo, may show other species to be paternal-brooders, enough is now known of the *Tilapia* fauna of East and Central Africa to say that the maternal-brooding habit is the general rule among *Tilapia* species in these waters. Aronson (1949) lists three other species, *T. dolloi*, *T. microcephala* and *T. simonis* in addition to *T. macrocephala* and *T. heudeloti* in which the male is credited with caring for the eggs, and the following in which both sexes have been reputed by some authors to care for the eggs, *T. simonis*, *T. galilaea*, *T. microcephala*, *T. nilotica* and *T. zillii*. There is conflicting evidence concerning the habits or systematic status of most of these species ; more recent work has made it clear that *T. zillii* is a substratum-spawner (Daget, 1952 ; El Zarka, 1956) ; *T. simonis* is now referred to a different genus (Aronson, 1949 footnote p. 134), and *T. microcephala*, *T. heudeloti* and *T. macrocephala* now appear to be synonymous (Boulenger, 1915, Trewavas 1947a). As nothing is on record concerning the ecology of *T. dolloi* Boulenger,* a Congo species studied by Asch, 1939 (referred to by Aronson, 1949), the behaviour of *T. macrocephala* (*T. heudeloti*) is here taken as the type behaviour for paternal-brooders.

* Also = *T. heudeloti* according to Trewavas, personal communication.

Tilapia species also fall into the same three groups on certain basic anatomical characters such as gill raker number. The substratum-spawners all have less than twelve gill rakers on the lower part of the anterior gill arch, whereas the maternal-brooders have more than fifteen (generally more than seventeen) and up to twenty-eight gill rakers on the lower part of the anterior arch, the number varying in different species. *T. macrocephala* (*T. heudeloti*) has an intermediate number, fifteen to nineteen, and *T. dolloi* twelve to fifteen, as do certain other West African and Congo species (Trewavas personal communication) among which other paternal-brooders may be found when more is known of their breeding habits.

II. SUMMARY OF DATA

A. *Breeding behaviour differences between the three groups of* Tilapia

A comparison of breeding habits of substratum-spawners with those of maternal-brooders in natural waters has shown the following differences in breeding behaviour associated with the two types of habit :

Substratum-spawners	*Maternal-brooders*
both sexes develop breeding colours and defend spawning territory	male only develops breeding colours and defends spawning territory
spawning pairs are generally solitary	male spawning territories are in colonies on definite spawning grounds
both sexes make nest	male only makes nest
long courtship preceeds spawning	very little courtship preceeds spawning, which is very rapid
male and female remain together while guarding young	female carrying eggs moves away from spawning grounds, so sexes segregate immediately after spawning
monogamy possible for several broods, sexes remaining together and preparing to spawn again as soon as young become independent	polygamy usual, male fertilising eggs from a succession of females which spawn in his nest
male and female take turns in guarding and feeding	female only broods and does not feed while doing so ; male feeds little while actively guarding nest
parents guard young near to where eggs were laid	brooding female seeks shelter of plant beds and may make long movements between spawning and brooding grounds
eggs and young remain on substratum for some time so oxygenated bottom water is necessary	eggs are only momentarily on the bottom, so poorly oxygenated bottom water may not limit spawning area

Aronson's studies on *T. macrocephala* suggest that the behaviour of the paternal-brooders is in many ways intermediate between that of the substratum-spawners and the maternal-brooders. In paternal-brooders there is little qualitative difference between the colour and behaviour of the sexes, both sexes help to make the nest (though this is primarily made by the female) and both sexes may brood (though this is generally carried out by the male). In *T. macrocephala* there is a courtship lasting several hours, or more often days, before spawning occurs, and Aronson (1949) points out that this is necessary to ensure exact synchronization of milt production with egg laying as *Tilapia* eggs water-harden very rapidly after they are laid. In maternal-brooders the male is able to fertilize continuously over a long period (several weeks or months), as the milt production cycle is not interrupted by guarding or brooding duties ; maternal-brooders spawn very rapidly with hardly any preliminary courtship (Lowe, 1956 a), this, and the fact that the eggs are picked up immediately by the female, may be a considerable advantage in the deoxygenated water on the bottom of water lily swamps where *Tilapia* often spawn. In *T. macrocephala* the male normally picks up the eggs, but should he fail to do so, or to pick up all of the eggs, the female may do so after a time lag of ten to twenty minutes ; so both sexes may brood. Paternal-brooders do not seem to have become so thoroughly adapted for mouth-brooding as have the maternal-brooders. For example, once the young are ejected by the male *T. macrocephala* they are never collected up again, and should they not find cover among the plants they are likely to be eaten by the parent ; the brooding fish eats little or nothing while incubating and is cannabilistic after the young are released, at least in aquaria (Aronson, 1949). In the maternal-brooders the young are collected up and brooded again when danger threatens and at night for a considerable period (about a week in many species) after they are first released from the parent's mouth. Again, in *Tilapia* the number of eggs laid at a time increases with the size of the female (Lowe, 1955 c) and in maternal-brooders the larger females which lay more eggs also have larger mouths in which to brood, whereas among paternal-brooders there is no such relationship between the size of the brooding mouth and the number of eggs produced. Aronson (1949) records that a small male spawning with a large female was not able to pick up all the eggs laid. This was, however, in an aquarium, nothing is yet on record about the relative sizes of the two sexes of *T. macrocephala* in natural waters and it is possible that the male generally spawns with a smaller female, as in the *T. mossambica* group of *Tilapia* mentioned on page 15. In *T. macrocephala* by the time the young are released the female is often prepared to spawn again ; in aquaria the interval between successive broods varies from eight days to a year with a mode at fifteen days (Aronson, 1945), and the presence of the male influences the spawning frequency of the female. In nature the male and female of paternal-brooders may not be so closely associated as they are in substratum-spawners in which both parents stay with the young.

B. *Ecological data*

The following differences in general ecology were noted between the three groups of *Tilapia* :

	Substratum-spawners	*Maternal-brooders*
Feeding habits :	*T. sparrmani* : unspecialised and omnivorous, using mainly bottom food	Mostly algal-feeders, using phytoplankton, epiphytic or bottom algae according to availability
	T. melanopleura, *T. zillii* : specialised for chewing aquatic plants	
Habitat :	restricted to shallow water where plants can grow	found in open lakes some distance from shore as well as in shallow waters of swamps and rivers
Movements :	restricted to plant areas for food and shelter so little movement about lake ; young live in shelter of plants near original territory	considerable movements occur between feeding and breeding grounds ; young are left in shelter of weeds and later move to open water

Very little is yet on record concerning the ecology of *T. macrocephala* but like the substratum-spawners this species appears to be restricted to shallow water areas where there are vascular plants which provide cover for the young and possibly food ; there are no very large lakes in West Africa and this species is said to inhabit brackish lagoons along the coast and the swampy deltas of the rivers (Aronson, 1949). Irvine (1947) says that the probable diet of this species is fine vegetable matter.

Thus all known *Tilapia* are primarily vegetable feeders, feeding on algae or aquatic plants, though occasionally zooplankters and insect larvae picked up with bottom debris are eaten. In L. Kivu *T. nilotica* feed on a large planktonic *Spirillum* for much of the year (Capart quoted by Fish, 1955), and in the Malagarasi swamps Protozoa in the thick bottom deposits may be an important source of food for *T. karomo* and *T. nilotica*. The substratum-spawning *T. melanopleura* and *T. zillii* appear to be the only *Tilapia* species in East and Central Africa which have become specialised for feeding on aquatic vascular plants. This uniformly vegetable diet in the genus *Tilapia* is in marked contrast with the very varied diet in the associated cichlid genus *Haplochromis* in which an extensive adaptive radiation has occurred and vegetarian, insectivorous, mollusc-eating, zooplankton-feeding and predatory species have evolved ; the significance of this difference between the two genera is discussed below.

Fish (1951 and 1955) drew attention to the fact that of the algae eaten by *Tilapia* the blue-green and green algae generally pass right through the fish undigested, and diatoms form the main usable food. Greenwood (1953) showed the roles of the mucus produced in the buccal cavity and the pharyngeal teeth in the feeding mechanism, and Gosse (1956) has shown that microgillrakers present on the inside of the gill arches also have an important role in the feeding mechanism of *Tilapia*.

Where several species of *Tilapia* occur in one lake they are often caught together and then generally contain the same food. This appears to be a contradiction of Gause's principle that two species with the same ecology cannot persist in the same region. Possibly phytoplankton is exceptional in being able to support closely related species* (factors other than food supply limiting the numbers of the fish), but these different species generally predominate in slightly different, though widely overlapping, zones, and they separate to breed (see below page 19). Thus in L. Nyasa the five species found at the southern end of the lake inhabit increasingly offshore openwater zones, from the substratum-spawning *T. melanopleura* which is restricted to lagoons and swampy areas round the lake, *T. shirana* which keeps to inshore water and does not make long movements, to three members of the *T. squamipinnis* complex, *T. saka*, *T. squamipinnis* and *T. lidole*, which occupy increasingly openwater zones and make increasingly long movements about the lake (Lowe, 1953). These latter three are often caught together and at these times contain phytoplankton, but when phytoplankton is scarce *T. lidole* make long movements to other areas, whereas the other two species may take to bottom feeding. The equilibrium between the relative numbers of these different species appears to depend on the fluctuating lake levels (Lowe, 1953). Similarly in L. Victoria *T. esculenta* and *T. variabilis* are caught together throughout the year in certain parts of the lake, and then contain phytoplankton ; but *T. esculenta* predominates in areas where the water is opaque and there are soft flocculent bottom deposits of algae, and *T. variabilis* predominates where the water is clearer and the bottom harder, off sandy and rocky shores, in rivers and in swamps around the lake (Lowe, 1956 b).

In L. Albert where four species of *Tilapia*, none of them endemic, are present there appears to be more variation in the food used by the different species. The substratum-spawning *T. zillii* feeds on aquatic plants around the margins of the lake ; *T. leucosticta* is found mainly in lagoons where it feeds on algae in bottom deposits ; *T. nilotica*, the largest-growing species, is the most ubiquitous and is found in lagoons in the estuaries of inflowing rivers and around the edges of the main lake, and it varies its food with the habitat, taking bottom deposits from the lagoons, epiphytic diatoms in the estuaries and epiphytic diatoms and plankton in the lake ; *T. galilaea* is found in the main lake off sandy shores and appears to be primarily a phytoplankton-feeder.

In the more uniform waters of L. George *T. nilotica* and *T. leucosticta* are the only *Tilapia* species, they are often caught together and then contain the same algal food (Fish, 1955) over most of the lake at any time of year. In this lake there is some indication that the numbers of *T. leucosticta* have increased since the selective fishery for the larger, and seemingly dominant, *T. nilotica* has been in operation (Lowe, 1958).

With the exception of the vascular plant-feeders which generally feed on algae when very small (de Bont, Deceuinck & Detaille, 1950 and personal

* Cf. observations by Fryer (1957, J. Anim. Ecol. **26**, 263) on the food of freshwater copepods ; herbivorous species do not appear to obey the 'Gausian hypothesis' which may be associated with superabundance of algal food.

observations), there appears to be little change in food eaten as the *Tilapia* grow, the small *Tilapia* feeding on small particles, algae and some small zooplankters, in the water where they are living. However, Le Roux (1956) found that in ponds in South Africa *T. mossambica*, *T. melanopleura*, *T. sparrmani* and *T. andersonii* less than 5 cm. total length all preferred zooplankton, from 5–8 cm. long they ate almost all available food, and above 8 cm. long took mainly the same food as the adult stages, *T. mossambica* and *T. andersonii* mainly phytoplankton, *T. melanopleura* plant material, and *T. sparrmani* was omnivorous with particular preference for bottom food ; (all these fish were evidently dwarfed by pond conditions). Algae occur in all the different zones occupied by *Tilapia* during the course of their life histories, and although some species are more specialised to take algae in one particular form (for example the phytoplankton-feeders have large mouths and very fine pharyngeal teeth) the morphological differences between species are very slight, and on the whole *Tilapia* are adaptable and will use whatever algae are most readily available, whether in bottom deposit, epiphytic or phytoplankton form.

The substratum-spawners are tied to plant zones as they both feed and breed among plants and show little movement from place to place. Among the maternal-brooders the more openwater-living species make the longest movements about the lake; this is particularly clear in L. Nyasa (Lowe. 1952). In L. Victoria marking experiments have shown that both *T. esculenta* and *T. variabilis* make long, often surprisingly rapid, movements ; fish of both species marked in the Kavirondo Gulf have been caught up to 150 miles away in Tanganyika waters of the lake, and other marked fish have shown movements of up to four miles (*T. esculenta*) and ten miles (*T. variabilis*) respectively in one day (Lowe, 1956 b). The reasons for these very rapid and long movements, and how often they occur, are not known. In all the maternal-brooding *Tilapia* in the Great Lakes regular movements are generally made (a) from feeding to spawning grounds, (b) by brooding females from spawning to brooding grounds, and (c) from zone to zone as the fish grow.

The most marked movements between spawning and brooding grounds occur in L. Nyasa, the greatest extreme being shown by *T. lidole* in which brooding females carrying eggs may move several miles from spawning to brooding areas, taking the young into relatively sheltered water along the shore (Lowe, 1952) ; these Nyasa species appear to have one, or at most two, batches of young in a restricted annual breeding season. In Victoria, on the other hand, females have several batches of young in succession (this seems to be the more usual course among *Tilapia*) ; brooding females are found with gonads starting to ripen again and such females probably do not move far from the spawning ground. The configuration of L. Victoria is such that there is generally a suitable shoreline nearby to which to take the young, so the distinction between spawning and brooding grounds is not as clear as in Nyasa and movements from one to the other are not so well-defined.

In both Nyasa and Victoria and in many other lakes the young *Tilapia* are left in the plant zone around the margin of the lake by the female ; they move out as they grow and are found in shoals along sandy shores when about 7–12 cm. in total length, and at 12–15 cm. are found in surface waters at some

distance from the shore. As they approach maturity (20–27 cm. long depending on the species) they frequent the bottom rather than the surface waters. This same pattern is followed by different species of maternal-brooding *Tilapia* in many of the Great Lakes. However, in Lake Albert (and possibly also in Lake Rudolf and Lake Tanganyika) 12–18 cm. long *Tilapia* are found inshore and some *Tilapia* remain inshore and in lagoons round the lake for all or most of their lives ; this appears to be associated with the presence of the predators *Lates* and *Hydrocyon* in these lakes (see below, page 16). In Lake Victoria 12–18 cm. long *T. esculenta* visit certain beaches at definite times each year (Lowe, 1956 b), these fish are still immature and the reason for this seasonal inshore visit of immature fish is not yet known.

Thus in the Great Lakes the maternal-brooding *Tilapia* inhabit many different zones, moving as they grow from swampy inshore areas, to waters off sandy shores, to open surface waters and then bottom waters some distance from the shore ; once mature they move from opaque waters where they feed to clearer water where they spawn, and to the shelter of plant beds to brood young ; there the fry are left by the female. Thus these *Tilapia* are not confined to distinct ecological zones. This and the long movements about the lake recently discovered by marking mean that there is little ecological or geographical isolation within a lake. The evolutionary effects of this lack of ecological and geographical isolation are considered below.

III. DISCUSSION

A. *The evolutionary significance of breeding behaviour and ecological differences between the three groups of* Tilapia

It is only among the maternal-brooders that extensive speciation has occurred ; all the endemic species of *Tilapia* in the Great Lakes are maternal-brooders. This is in spite of the fact that the substratum-spawners are probably nearer to the basal stock from which the *Tilapia* have evolved, hence more ancient, as both the morphological characters, such as the low gill raker number, and the wide distribution of the few species suggest ; also, many of the South American cichlids are substratum-spawners. In *T. sparrmani* the structure, relatively small size and unspecialised omnivorous bottom-feeding habits, suggest that of the present day living *Tilapia* this species is probably nearest to the basal stock from which the *Tilapia* evolved. The wide distribution of *T. melanopleura* and *T. zillii*, right across Africa from the Gambia to the Nile, and south as far as Southern Rhodesia in the case of *T. melanopleura*, also suggests that these are relatively old species. But despite the apparent age of these substratum-spawners they do not appear to have speciated at all in East and Central Africa.

Paternal-brooders are, as far as is known, restricted to West Africa and the Congo and have evidently not undergone much speciation. Their behaviour is in some respects intermediate between that of the substratum-spawners and the maternal brooders, as mentioned above, also their gill raker number is intermediate. Two possibilities are presented concerning the relationship between the paternal- and maternal-brooders, either (1) the maternal-brooders

originated from paternal-brooding species in or near West Africa or the Congo, and the paternal-brooders could not compete with the more specialised and efficient maternal-brooders in colonising new areas, or (2) the paternal-brooding and maternal-brooding habits have arisen independently from the substratum-spawning habit ; if so, they may be of comparable antiquity, or the paternal-brooding habit may have arisen more recently which might account for its more limited distribution. Data are as yet insufficient to decide which surmise is correct ; the intermediate gill raker number of the paternal-brooders would appear to be in favour of the first alternative, but on the whole the differences in behaviour suggest the independent origin of the two groups from substratum-spawners. Substratum-spawners pick up the eggs and young in the mouth when moving them from place to place. Breder (1933) and Myers (1939) when discussing the evolution of oral incubation in fish point out that the habit has arisen many times independently, and in the family Cichlidae this would certainly seem to have occurred.

1. *Characters associated with the extensive speciation of maternal-brooders*

(a) *Preadaptation for life in openwater*

Maternal-brooders both by their ability to use planktonic algae and by their habit of brooding young are independent of the shoreline for much of their life and so are preadapted to exploit more openwater conditions. In Africa the formation of large lakes and alternation of arid and pluvial periods has presented unpopulated openwaters to the fish which could take advantage of them, as Worthington (1937) and Trewavas (1949) have pointed out, and the maternal-brooders were pre-eminently suited to do this. All the endemic *Tilapia* of the large lakes are maternal-brooders, as are also the other species such as *T. nilotica* and *T. galilaea* living offshore in the Great Lakes, as well as all the other cichlids in the Great Lakes whose breeding habits are known.

The ability to cross openwater combined with the necessity of moving inshore in a deep lake to find bottom suitable for spawning might, provided the openwater was not crossed very often—and in the early days of adaptation to openwater crossings would probably have occurred less frequently—lead to the establishment of discontinuous populations. Also, the female does not feed while brooding and might then cross more openwater, poor in phyto-plankton, than that visited by the fish at other times. Thus the female's wandering movements from spawning to brooding grounds might lead her into new arms or bays where she would leave her young and the area would be colonised by young of one stock, partially isolated from other populations. The effectiveness of this isolation would depend on how frequently fish crossed to the new area and whether the fish within it remained there to breed. Close inbreeding would lead to the more rapid establishment of any mutations throughout the population. Such movements may have been important in the evolution of endemic *Tilapia* species within Lake Nyasa, as is discussed below (page 25). Recent introductions of *Tilapia* into new areas in many parts of the world have shown how very rapidly whole new areas become populated from very few fry, provided not too many predators are present.

(b) *Polygamy*

The maternal-brooders are polygamous whereas among the substratum-spawners monogamy, at least for several broods, may be the general rule. The male of the maternal-brooders fertilises as many females as he can lead to his nest, and as many males congregate on the spawning grounds a particularly successful male may spawn more often than other males in the vicinity. Kosswig (1947) suggested that " monogamy " may have been important in the rapid speciation of cichlids in the African lakes, but it is the polygamous species, and not the monogamous ones, which have speciated ; Kosswig assumed that the cichlids of the Great Lakes had the " father-and-mother " type of parental care, i.e. were not mouth-brooders, whereas it is now known that the endemic cichlids of the Great Lakes are all, as far as is known, maternal-brooders. The ability of a particularly successful male to fertilise eggs from more females than do other males may have had important effects in spreading characters in a population. The presence of many males together on the spawning grounds presents ideal conditions for sexual selection to operate, and this would appear to have contributed to the development of the very striking breeding colours shown by the males of the maternal-brooders. A female normally spawns one batch of eggs all in one nest ; whether she ever moves on to another nest if interrupted while spawning, and then rears a brood fertilised by more than one male, needs further investigation.

2. *Characters of substratum-spawners which may have inhibited speciation*

The Cichlidae appear to be a relatively young family of fish—in East Africa the earliest fossils are probably those of Miocene age studied by Greenwood (1951 b)—and still in a very plastic state, speciating freely whenever conditions are suitable. It is therefore perhaps important to consider what characters may have inhibited speciation in the substratum spawners. These would seem to include the fact that they are limited to shallow waters by food and spawning requirements ; they have little incentive to more long distances as feeding and breeding grounds are close together, so that extensions of range will take place along shore lines and through swamps (which will account for their wide distribution). Under such conditions the new breeding populations will be contiguous with the parent population. Although it has been argued that distance and the presence of intervening populations along a shore line would promote isolation and speciation if the fish did not move much, the fact remains that those *Tilapia* which are restricted to inshore areas—both the substratum-spawners and the maternal-brooding *Tilapia* in lakes where these are restricted to inshore waters by predators (see page 16)—have not speciated. Local differences between populations do arise among substratum-spawners, but do not attain specific rank significance, perhaps because in the shallow waters in which these species live environmental changes, such as fluctuations in water level, are likely to be more rapid than in deeper bodies of water, and are unlikely to allow time for the development of new species. The substratum-spawners are likely to be monogamous at least for several broods ; the effect of this is difficult to see, as discussed above Kosswig (1947) thought that this would promote speciation, but it is just the monogamous species which have not speciated.

In the substratum-spawners both the type of food eaten and the absence of brooding habit bear a close relationship to the low number of gill rakers. The few widely spaced gill rakers, together with the course pharyngeal teeth which allow the consumption of relatively large food particles, have led away from the plankton-feeding habit in these species. Substratum-spawners lay smaller eggs than do maternal-brooders (Lowe, 1955 c). Although the eggs and young, which are also very small, are on occasion moved from place to place in the parent's mouth, trials have shown that the small eggs pass quite easily through the widely-spaced gill rakers. This combination of few gill rakers and small eggs makes mouth brooding impossible and the loss of eggs and young through the gill rakers would presumably militate against the evolution of the brooding habit in these species. Although the evolution of lakes is, in general, towards less openwater conditions as the lake matures, these African Great Lakes have offered greater opportunities to fish which could exploit more openwater conditions. In the substratum-spawners the interrelationships of gill raker density, food and spawning habits have evidently prevented these species from taking advantage of these more openwater conditions.

3. *The significance of breeding colours and associated characters*

Experimental work by Baerends & Baerends-van Roon (1950) on *T. mossambica* in aquaria suggested that the colour of the breeding male is very important for successful spawning in these maternal-brooding *Tilapia*, and it was of great interest to find that the two morphologically almost identical *Tilapia* in Lake Nyasa, *T. saka* and *T. squamipinnis*, developed different coloured breeding males (pitch black sometimes with white edges to the dorsal and caudal fins in *T. saka* and light blue with a white head in *T. squamipinnis* (Lowe, 1953)). This suggested that the male breeding colour was important for species recognition by the female and perhaps an important part of the isolating mechanism between closely related sympatric species. Since then much more has become known about breeding colours in other *Tilapia* species. These data suggest that there are certain trends in breeding colours which may in some cases be a guide to systematic relationships, and that the possession of such different breeding colours in two such closely related species as *T. saka* and *T. squamipinnis* is quite exceptional. Before discussing these breeding colour trends it is necessary to consider the possible functions of the breeding colours and whether these functions are the same in all three groups of *Tilapia*.

Breeding colours and their functions in the three groups of Tilapia. The paternal-brooder *T. macrocephala* does not develop any special breeding colours in the ripening fish ; in most populations there is, however, a secondary sexual difference in the presence or absence of pigment in the operculum of the uncastrated fish (Aronson, 1951). In the substratum-spawners the ripening fish of both sexes develop similar breeding colours and guard territory ; their colours vary very much with the activity of the fish (and they generally fade on death), they become intensified when they are driving away other fish, indicating that they have a function of territory advertisement, and they also assist

courtship in the synchronisation of the male and female cycles. In the maternal-brooders only the ripening male develops breeding colours and establishes and guards a territory, and these colours are lost again by the spent fish. The female retains the drab colours of the non-breeding fish which are presumably of protective value (in association with her habit of swimming inconspicuously with her dorsal fin down) when hiding among weeds brooding young, though in some species, such as *T. leucosticta*, the branchio-stegal membrane may become deeper in colour and the pelvic fins darker.

The male breeding colours of the maternal brooders serve for advertisement of spawning grounds, an effect which is enhanced by the habit of many males congregating together in certain places to spawn. This advertisement effect is most marked among the species endemic to Lake Nyasa where the breeding season is restricted ; in this lake the males congregate on well-defined spawning grounds and have very definite breeding colours (black in several species) which show little variation in intensity throughout the short breeding season (and are retained in the dead fish). In Lake Victoria where some ripe *Tilapia* are found at all times of year breeding places are not so well-defined and male breeding colours are not so intense ; in aquaria the colours of the Victoria *Tilapia* show great variations in intensity with the activity of the fish (and half-fade on the death of the fish).

Advertisement of spawning grounds is particularly necessary in Nyasa as, in addition to the restricted breeding season, the spawning grounds may be some way from the feeding grounds inhabited by the fish at times of year when they are not breeding. This is because each species tends to spawn in the most clear water zone of its range, where the sandy bottom provides a good substratum for nest making, and to feed in the most eutrophic part of its range as this carries the best phytoplankton (Lowe, 1953). In Lake Victoria there are not such sharp distinctions between feeding and breeding areas, possibly because the water inhabited by the *Tilapia* in this relatively shallow lake is more uniform than in Nyasa.

In the maternal-brooders male breeding colours may also have an important role in species recognition and in stimulating the female to lay. In these species the pre-spawning courtship is very short, the ripe females wander from nest to nest then suddenly spawn in one nest, sometimes without any preliminary courtship. (In *T. variabilis* which has two colour forms the atypical piebald-and-orange form is almost limited to the females (occurring in over 30 per cent of females but in less than 5 per cent of the males) and no ripe males of the atypical colour form were ever found (Lowe, 1956 b).

It should be mentioned in passing that in the maternal-brooders nest forms as well as breeding colours and associated behaviour may also be important for species recognition and release of egg laying, as the nests often have a characteristic form in different species, although the form may vary somewhat with the substratum. In *T. karomo* the nest is a small plaque on a high mound (Lowe, 1956 a), in *T. macrochir* the nest has radiating grooves leading out from a central crater (figured by de Bont, 1950), and the nests of Pangani species and *T. variabilis* have small pits around the central crater (Lowe, 1955 ;

1956 b). (In the substratum-spawners an area of rock or stick is cleared for the adhesion of the eggs, and holes are prepared into which the minute young, which have adhesive head glands are transferred).

Thus in both substratum-spawners and maternal-brooders the breeding dress, assumed by both sexes in the former and by the male only in the latter, advertises the spawning territory, and among the maternal-brooders the congregation of many males on the spawning grounds enhances this effect. In substratum-spawners breeding colours may also aid courtship in synchronising milt production with egg deposition (though this synchronisation is achieved without breeding colours by the paternal-brooders), whereas in the maternal-brooders milt is produced over a long period, courtship is extremely short and male breeding colours, possibly in conjunction with nest form, may be important for the recognition of the species by the female and in stimulating the female to lay.

Breeding colour trends. In certain maternal-brooding *Tilapia* it seems that breeding colours may indicate systematic relationships. In Nyasa, despite the apparent and probable importance of the different male breeding colours for species recognition in *T. saka* and *T. squamipinnis* mentioned above, most of the *Tilapia* species have black breeding males. *T. lidole* and *T. karongae*, like *T. saka*, have black breeding males, sometimes with white tips to the dorsal and caudal fins, and these species seem to depend on other morphological differences and differences in spawning place to keep separate. Thus black seems to be the basic colour pattern for breeding male *Tilapia* in Nyasa and in this the *Tilapia* are distinguished from other cichlid genera in the lake (including *Corematodus shiranus* Boulenger which mimics and lives with the shoals of *Tilapia* and appears to feed by rasping scales from their tails (Trewavas, 1947 c) but which develops a blue and old gold breeding dress). In the Pangani river system the males of the two species endemic to Lake Jipe and *T. pangani* from the river, all closely related species, have very similar male breeding colours (Lowe, 1955 a). *T. esculenta* which replaces *T. nilotica* in the Nile system above the Murchison Falls, and *T. nilotica* both have a black and red male breeding dress, unlike that of any other known *Tilapia*, which is almost identical in the two species (Lowe 1958) and supports the view that these geographical replacement species are closely related, as well as ecological counterparts in their respective waters.

There is, however, a common form of male breeding dress, with a greenish-blue body and strongly contrasting bright orange edges to dorsal and caudal fins and sometimes with an orange or white genital papilla or tassel, which is found in many species of *Tilapia* in widely separated parts of East and Central Africa, and it is not yet clear whether bearers of this dress form one group of closely related species or whether this breeding dress has arisen independently several times. The breeding colours of *T. variabilis* in Lake Victoria, *T. karomo* in the Malagarasi, the Lake Jipe and Pangani river species, and *T. andersonii* in Southern Rhodesia all approximate to this pattern. This is a highly functional colour pattern, the orange edges to the fins when the fins are extended making the male look larger and more conspicuous when guarding

his spawning territory (Lowe, 1956 a)—a function which the white edges on the fins of the Nyasa species may also serve—whereas the blue-green body colour renders the fish relatively inconspicuous as he flees from his territory with fins down if suddenly disturbed (Lowe, 1956 a).

Increasing the apparent size may be important as Baerends & Baerends-van Roon (1950) found that in *T. mossambica* in aquaria the size of the male was important in allowing him to stake and keep a spawning territory. In nature space is not so limited, but in some places the nature of the bottom and deoxygenation of the bottom water may limit the area of suitable spawning ground (Lowe, 1956 a). In *Tilapia* of the *T. mossambica* group, *T. mossambica*, *T. nigra* and some Pangani river species, the males are nearly always bigger than the associated females ; these are species which have evolved in rivers, where suitable bottom for spawning may be limited and the size of the male at a premium for successful spawning. Experimental work in ponds has shown that the males of these species actually grow faster than the females (Vaas & Hofstede, 1952, Brown & van Someren, 1953 ; Lowe, 1955 a), and in these species the mature male develops a characteristic enlarged mouth and concave upper profile, so the growth characteristics and the appearance of the adult fish are considerably different in the two sexes. In the *Tilapia* species endemic to the Great Lakes the males and females grow to, and mature at, comparable sizes and retain the same general head shape, and pond experiments have shown that they grow at the same rate. Though the full significance of this differential growth rate in the two sexes is not yet clear, this character seems to be genetically determined and of significance for the study of the systematic relationships between different groups of *Tilapia*.

4. *Environmental influences on speciation*

Thorpe (1945) in a paper considering the evolutionary significance of habitat selection has stressed the importance of imprinting in the young of birds and insects in determining where they will return to breed. In the maternal-brooding *Tilapia* the female carries the eggs away from spawning to brooding grounds and the first impression of the young fish will be of quite a different ecological zone than that to which they will later go to spawn ; also the young will not see the breeding male. Hence it seems that under natural conditions knowledge of the nature of the spawning grounds, and of the colour of the breeding male, cannot be due to imprinting in these species, though in the substratum-spawners, in which the young follow both parents in the same environment in which they will later spawn, the learning mechanism may be different.

The environmental factors most concerned in speciation are those which present barriers, physical or temporal, between breeding populations, whether in different waters or within one body of water. The differences between Lakes Nyasa and Victoria discussed in a later section (page 21) illustrate some of the barriers which may operate within one body of water and these are therefore considered in this later section. A *Tilapia* changes its environmental niche many times as it grows and different factors may operate at the various stages of its life-history. In addition to environmental and behaviour

barriers which may allow an incipient species to become a new species, biotic factors such as predation pressure have been thought to influence speciation by affecting the numbers of the incipient species which survive.

Worthington (1937, 1940, 1954) maintained that predator pressure was an important factor in controlling speciation, since incompletely adjusted forms would be selected against when predator pressure was high. Worthington was greatly impressed by, and recent work has confirmed, the striking differences between the faunas of Lakes Albert and Rudolf, which have the predatory *Lates* and *Hydrocyon* and hardly any endemic cichlids, and those of Lakes Victoria, Edward-George and Nyasa, which are without these predators and have numerous endemic cichlids. Lake Tanganyika presents rather a special case since it has these predators and a higher proportion of endemic cichlid genera than any other lake ; the probably greater age of this very deep lake has been invoked to explain this (Worthington, 1940 ; Trewavas, 1949). Worthington (1932) used as evidence of differential predator pressure his observation that fish with abnormalities were not found in lakes with these predators, Albert and Rudolf, whereas they are quite commonly found in the other lakes, Victoria and Edward-George, an observation confirmed by more recent study ; from this Worthington inferred that such abnormal fish could not survive in lakes where the predator pressure was high.

Fryer & Isles (1955) suggested that the restrictive effect of predators on evolution has not been so important as Worthington believed as Nyasa, though lacking *Lates* and *Hydrocyon*, has a large number of predatory cichlids and several predatory non-cichlids (species of *Bagrus*, *Clarias*, *Barilius* and *Barbus*). Predatory *Bagrus* and *Clarias* are also found in other lakes without *Lates*, such as Victoria and Edward-George, in which extensive speciation has occurred. Most of the predatory cichlids in Nyasa are, however, small, and *Bagrus* and *Clarias* appear to be " lurking " rather than " swift " predators compared with *Hydrocyon*. Much remains to be learnt of the relationships between the various predators and prey in all these lakes before it is possible to draw conclusions regarding the role of predators in speciation.

Recent work has, however, stressed that in Lakes Albert and Rudolf the fish of many genera are much larger than their counterparts in the other lakes and the *Tilapia* tend to have longer stronger spines (personal observations). Also, the distribution of the *Tilapia* is different, the *Tilapia* being restricted to the edges of the lake throughout all or most of their lives in the lakes with *Lates* and *Hydrocyon* and having openwater-living phases in the other lakes ; this also applies to Lake Tanganyika where *T. tanganicae* is the only really lacustrine species but even this species is restricted to the littoral zone, to water less than 10 metres deep along sandy shores (Poll, 1956). Thus the particular predators *Lates* and *Hydrocyon*, or some associated factor, do seem to have had a marked effect on the fish, and they might conceivably have had effects on evolution by controlling the distribution of the fish within the lake. In the case of Lake Tanganyika, which though evidently an older lake than Nyasa has no species flock of *Tilapia*, these predators may have restricted evolution within this genus.

It is, however, among the *Haplochromis* and not among the *Tilapia* that the difference between lakes with and without *Lates* is most marked. *Tilapia* provide little evidence as there is little difference in the number of species between lakes with and without *Lates*, as the following Table 1 shows. Also as is discussed in a later section, Nyasa is the only lake with a genuine species-flock of *Tilapia*, and predators are likely to have most effect on the sympatric development of species flocks as these involve the greatest changes in the habits and habitats of the fish.

TABLE 1

The numbers of *Tilapia* species in lakes with (+) and without (—) the predators *Lates* and *Hydrocyon*

		Tilapia species	
Lake	*Lates* and *Hydrocyon*	*No. present*	*No. endemic*
Rudolf	+	3	0
Albert	+	4	0
Tanganyika	+	4	2
Edward-George	—	2	0
Victoria	—	2	2
Nyasa	—	6	5
Rukwa	—	1	1
Jipe	—	2	2

Lakes which already have several species of *Tilapia* do not develop endemic species unless the *Tilapia* can exploit more openwater (as they have done in the case of Nyasa) ; this is probably bound up with *Tilapia's* restriction to a vegetarian, mainly algal, diet, and suggests that interspecific competition in inshore waters, as well as presence or absence of openwater predators, may play a part in controlling the distribution and further evolution of *Tilapia* species.

Can interspecific competition between different species of *Tilapia* have affected their further evolution ? Data summarised above concerning the food habits suggest that competition for food is not often a limiting factor in these algal-feeding fish under natural conditions, even though several species may use the same source of food for much of the year. It is, however, possible that there is competition for spawning grounds in lakes such as Victoria where suitable hard bottom appears to be limited, and for nursery zones, as so much of the shallow water in the swampy areas at the edge of the lake, used as a nursery zone by the 3–10 cm. long fish of both Victoria species, is unavailable because it is deoxygenated. The distributions of *T. esculenta* and *T. variabilis* in Lake Victoria, and of *T. nilotica* and *T. leucosticta* in Lake Edward-George suggest that inter-specific competition exists, the larger-growing *T. esculenta* and *T. nilotica* dominating the other two species and affecting their distribution in their respective lakes (Lowe, 1956 b and 1958) ; such a situation could presumably have effects on the future evolution of the fish by governing their distribution within the lake.

B. *The significance of the Lake Nyasa* Tilapia *species flock*

Several lakes, Nyasa, Victoria, Tanganyika and Jipe, have more than one endemic species of *Tilapia*, but Nyasa is unique in having more than two endemic species (Table 1). Endemic fish species arise either by gradual change

in a population isolated from other water systems (allopatric speciation) or by speciation within a lake (sympatric speciation). In Nyasa four species form a genuine " species flock " in the sense defined by Brooks (1950) as a flock of species in a lake all more closely related to one another than to any population existing outside the lake. No similar *Tilapia* species flock is found in any other of the lakes, despite the fact that large *Haplochromis* species flocks also occur in Lakes Victoria and Edward showing that conditions were suitable for the extensive speciation of some cichlids in these lakes too. The unique Nyasa species flock presents an opportunity to examine the ecological conditions, and their counterparts in the behaviour of the fish, which may have led to the evolution of a species flock in Nyasa and not in these other lakes.

1. *The sympatric speciation controversy*

Much has been written on the subject of whether sympatric speciation has occurred among cichlids in the African lakes by Worthington (1937, 1940, 1954), Mayr (1942, 1947), Trewavas (1941, 1947 b, 1949), Brooks (1950), and for *Haplochromis* species by Greenwood (1951 a). The gist of the controversy is that Worthington after extensive field studies in East Africa held that fish species in the Great Lakes had arisen by adaptive radiation within the lakes. Mayr (1942) maintained, largely from his work on birds, that geographical isolation was always necessary for the formation of new species and that new fish species could not arise sympatrically (then taken to mean within the same lake), and he suggested that multiple invasions had been more frequent in the African lakes than realised by those who had worked there. Much turned on the exact meaning of the word " sympatric " and in later papers Mayr (1947) somewhat modified his view on the scale of isolation necessary, evidently partly as a result of Thorpe's work (Mayr, 1947, page 266) as Thorpe (1954) pointed out, primarily from work on insects, that changes of ecological preferences often preceed geographical isolation. In recent years it has become evident that ecological isolation may entail microgeographical isolation (see Poll, 1952 ; Worthington, 1954), and that many of the species present in a large lake, and thereby " sympatric " according to Mayr's earlier (1942) view are in fact ecologically and microgeographically isolated. This applies, however, particularly to species which remain in one ecological zone throughout life, as do many of the *Haplochromis* species, but not so much to *Tilapia* species which move from zone to zone as they grow.

The *Tilapia* species flock has figured prominently in this controversy and the position concerning these species has been admirably summarized by Trewavas (1949). In Nyasa there are six species of *Tilapia*, the non-endemic *T. melanopleura*, the endemic *T. shirana* which is most closely related to *T. mossambica* found in the Shiré river flowing from the lake, and a species flock of four endemic species, *T. squamipinnis*, *T. saka*, *T. lidole* and *T. karongae*, which are all more closely related to one another than to any species outside the lake. These four species differ from one another largely in the degree to which they are adapted for openwater conditions, indicating that they have evolved under such conditions. All the evidence from morphological, ecological and behaviour studies, and from studies of the history of

the lake, leads to the conclusion that these four species have evolved within the lake. Trewavas (1949) pointed out that lake level changes may have added to the possibility of isolation in bays and creeks as suggested by Mayr (1947), but she concluded that in Nyasa geographical isolation appears to be supplemented by " minutely specialised breeding habits, induced or selected by environmental change ". Brooks (1950) when reviewing the question of speciation in ancient lakes concluded that in the unparalleled evolution of cichlids in the African Great Lakes there is little doubt that biological factors are acting to reinforce the initial isolation controlled by intralacustrine geography ; he referred to Kosswig's (1947) suggestion (without, however, realising that Kosswig's assumption that the Great Lakes cichlids are monogamous was wrong, see page 11) that specialised breeding behaviour may be important in restricting genetic interchange, and he concluded that any genetic divergence in a population isolated for only a relatively short time might, particularly if the colour pattern were altered, lead to inbreeding and more or less complete reproductive isolation even when the geographical barriers are no longer effective.

The question now is how much have geographical and ecological isolation within the lake been concerned and how much have other factors such as breeding habits contributed to speciation within Lake Nyasa. The widely overlapping zones occupied by the three species of the *Tilapia* complex which live at the southern end of the lake, where they live in mixed shoals for much of the year, have been considered above (page 7). Apart from *T. karongae*, which appears to be restricted to the northern half of the lake where it is found together with the other species, there is no evidence of any present-day geographical isolation, or of any complete ecological isolation among the present day *Tilapia* species.

Conditions for the formation of a new species demand (a) colonisation of new areas, and (b) isolation, or semi-isolation until the genetic characters become fixed. Ecological isolation need not, however, be complete throughout life, provided that the different populations are isolated from one another when the fish are breeding. This principle is particularly well demonstrated in the Nyasa *Tilapia* and does not seem to have been generally appreciated ; (it is very relevant for the many studies of the operation of Gause's principle (see page 7) as this is generally interpreted in terms of competition for food). In Nyasa shoals of different *Tilapia*, *T. saka*, *T. squamipinnis*, and *T. lidole* are caught together, all feeding on phytoplankton, for much of the year, but differences in depth and place at which the different species spawn ensure spatial isolation when spawning. This spatial difference is reinforced by temporal isolation as there are well-defined breeding seasons, and differences in colour of the breeding male (see page 12) ; this Nyasa species flock will be considered further (page 24) after more detailed examination of some of the factors which may have affected speciation in Lake Nyasa.

2. *The uniqueness of the Nyasa* Tilapia *species flock*

Reconsideration of the Nyasa *Tilapia* species flock in the light of further knowledge of the ecology and behaviour of cichlids in East African lakes

stresses that Nyasa is unique in having a *Tilapia* species flock and that this species flock is very small compared with the *Haplochromis* species flocks in this and other lakes. In Nyasa there are only six species of *Tilapia* compared with over a hundred recorded species of *Haplochromis* and many other cichlids of different genera (Bertram, Borley & Trewavas, 1942). In Victoria there are only two species of *Tilapia* compared with over seventy closely-related species of *Haplochromis* (Greenwood, 1954). This paucity of *Tilapia* species compared with *Haplochromis* species is probably connected with (a) *Tilapia's* restriction to algal food, whereas among the *Haplochromis* there has been an extensive adaptive radiation exploiting every available kind of food (see page 6), and (b) *Tilapia's* habit of moving from zone to zone in the lake as the fish grow and also making long movements in the lake. Thus there is little ecological or geographical isolation between populations of *Tilapia*. This is in direct contrast with *Haplochromis* species, which are all smaller than the *Tilapia* and tend to remain in one zone (for example over a hard substrate, or over mud, or sand, or in plant beds, or where the shoreline is rocky), throughout life (Greenwood, 1954), and are therefore, unlike the *Tilapia*, split up into populations which are subject to a high degree of isolation.

The only other lakes at present known to have more than one endemic species of *Tilapia* are Tanganyika, Victoria, and Lake Jipe (a small lake on the Pangani River), which each have two endemic species (Table 1). In Tanganyika the two endemic species, *T. tanganicae* and *T. karomo*, appear to have evolved under very different conditions and in different areas, the former in the main lake and the latter in the Malagarasi swamps flowing into the the lake ; the two species only meet in the Malagarasi delta so there is geographical as well as ecological isolation. In addition, these two species appear to have come from different stocks, *T. karomo* from the *T. mossambica* group and *T. tanganicae* from the *T. galilaea* group of *Tilapia*. In Lake Jipe and Lake Victoria the two endemic species concerned are found together in each case, but it appears probable that in Lake Jipe two invasions have occurred and that in Lake Victoria the two species may have come from different stocks of *Tilapia*.

Lake Jipe is a shallow lake, probably not more than ten metres deep at the present day. With the well-known fluctuations in rainfall in East Africa this lake must have fluctuated greatly in extent and must have more or less dried out several times and then been recolonised from the Pangani River. Of the two endemic species *T. girigan* is very little different from *T. pangani* now found in the Pangani River, and *T. jipe*, which is more distinct, may represent the result of an earlier invasion from the river. This interpretation is supported by the similar male breeding colours and nest forms found in these two endemic species and in *T. pangani* (Lowe, 1955 a). The mechanism whereby these two sympatric species keep distinct is not yet known.

Trewavas (1949) and Worthington (1954) considered that *T. esculenta* and *T. variabilis* in Lake Victoria might be a species pair, but more recent field studies on *Tilapia* in East Africa lead to the conclusion that these two Victoria species are more distinct than was formerly believed. Certainly they are more distinct than are the members of the Nyasa species flock, and in the

field *T. variabilis* gives the impression of being closer to the *Tilapia* of the eastward-flowing rivers allied to *T. mossambica*, and *T. esculenta* to *T. nilotica*, although Trewavas (1949) considered that they both could have evolved from one *Tilapia* not very different from the population named by Günther *T. spilurus*, now existing in the eastward-flowing Tana River in Kenya. Greenwood (1951 b) considered that Miocene fossils from Lake Victoria appeared to be most closely related to *T. mossambica* Peters. Greenwood (1951 a) has summarised ideas concerning the history of Lake Victoria and postulated that the fish retreated into the east-west river systems as the lake more or less dried up in the interpluvial periods, and then recolonised the lake as it expanded again during the pluvials, and it seems possible that the two present-day *Tilapia* may have originated from stocks entering the lake at different times or from different rivers.

3. *The contrast between* Tilapia *speciation in Lakes Nyasa and Victoria*

(a) *The effects of depth and age of the lake*

There is no evidence of any complete geographical isolation in Nyasa and the configuration of the present shoreline would make this seem improbable. However, the Nyasa *Tilapia* although adapted for " openwater-living " are rarely found over water more than fifty metres deep, probably because phytoplankton is less abundant there, and such deep water may form a barrier which is infrequently crossed. In Nyasa deep water comes right inshore at several critical places, for example between the south-east and south-west arms of the lake. The frequency with which such barriers are crossed is likely to be of significance. Also, as suggested above, such barriers may be crossed by brooding females, which are then not feeding, moving to inshore areas with their young. In the shallower Lake Victoria, conditions are more uniform and there is less necessity for the fish to move right inshore to find shallower water for spawning, so breeding populations are less likely to be discontinuous than in the deeper Lake Nyasa. In Victoria the *Tilapia* disappear from the catches at the time when phytoplankton is blooming in the main lake and it is possible that there are some seasonal movements into the open lake although very few *Tilapia* are caught out there ; marking experiments mentioned above (page 8) have shown that some Victoria *Tilapia* make long movements about the lake. The only exception noted to the lack of geographical isolation in Lake Victoria was the collection of a few rather atypical *T. variabilis* from near Godziba Island right out in the centre of the lake ; possibly this Godziba population has not been isolated, or sufficiently isolated, for long enough, to have become specifically distinct. The fluctuations in level known to have occurred in all these African Great Lakes would lead to greater mixing of populations when the level was low in the more uniform and shallower waters of Victoria than in the deeper waters of Lake Nyasa.

Lake Nyasa is thought to be a much older lake than Victoria so there has probably been longer for semi-isolated populations to become specifically distinct. The coefficient time x degree of isolation is undoubtedly important. Carter (1951, page 190) summarized work by Wright and others or the

mathematical effects of isolation in populations of various sizes, and concluded that conditions for evolution are most favourable in medium-sized local populations partially isolated from similar populations of the same species. In Lake Victoria there has been time for the evolution of a large *Haplochromis* species flock, but speciation in this genus is likely to have been more rapid than in *Tilapia* owing to the high degree of ecological and microgeographical isolation in the former genus, as discussed above (page 20). This emphasises that the degree of isolation, largely determined by the habits of the fish, has evidently been of far greater importance in the evolution of new cichlid species than the age of the lake *per se*.

(b) *The effects of defined breeding seasons*

In Lake Victoria some ripe *Tilapia* are found at all times of year. In Nyasa the *Tilapia* have well-defined breeding seasons and different species breed at somewhat different times of year. This means that temporal isolation is added to spatial isolation between species. The most marked difference is between *T. saka* which breeds in the hot weather before the rains, August to November, in water 2–4 metres deep, and *T. squamipinnis* which breeds during the rains from December to April, in water 16–20 metres deep (Lowe, 1952).

The presence or absence of defined breeding seasons among *Tilapia* depends on the hydrological conditions and these are primarily dependent on the geographical latitude. Lake Nyasa lies between 9° and 15° south and has a well-defined annual hydrological cycle in accordance with annual wind and rainfall cycles (Beauchamp, 1940). In the equatorial Lake Victoria the hydrological cycle is less definite and differs in the main lake and the Gulfs (Fish, 1957), and the *Tilapia* can escape an annual though ill-defined cycle in one part of the lake by moving about the lake, as marking experiments have shown that they do. The effect of latitude and hydrological conditions on *Tilapia* breeding seasons is clearly shown in those species found over a wide latitudinal range. Thus *T. nilotica* in Northern Nigeria (about 10° to 13° north) have a restricted breeding season, breeding only in the rains, whereas in equatorial waters breeding fish are found at all times of year (Lowe, 1958). Similarly *T. mossambica* in South Africa and Rhodesia breed only in the warm season (du Plessis & Groenewald, 1953 ; Jubb, 1952 ; Maar, personal communication) but in Indonesia (introduced) breed throughout the year (Vaas & Hofstede, 1952). Among many *Tilapia* most ripe fish are found in the rainy seasons, for example among *T. macrochir* and *T. melanopleura* in ponds in the Congo (de Bont, 1950), *T. rukwaensis* in Lake Rukwa (Ricardo, 1939 b, Swynnerton unpublished report), and Lake Victoria species (Lowe, 1956 b), but Aronson (1957 p. 274) records that in *T. macrocephala* in West Africa there was a marked drop in the spawning frequency during the period of heavy rains, coupled in some areas with retrogression of the gonads. How the effect of the rain operates is not clear ; Aronson (1957) suggests that intensity of illumination may be a controlling factor. In Nyasa *T. saka* and *T. lidole* both breed before the rains and *T. squamipinnis* during the rains (is it significant that the latter species, which spawns deeper, spawns after a longer period of light increase ?) ; *T. karongae* at the northern end of the lake and

T. shirana which lives inshore both appear to have more extended breeding seasons.

The Nyasa species have more restricted breeding seasons than species on a nearly comparable latitude in Rhodesia, where *T. mossambica*, *T. macrochir* and *T. andersonii* start to breed as soon as the " weather becomes warmer " about September and continue to spawn throughout the warm weather until about March, having many successive broods. The very restricted breeding seasons in Nyasa appear to be exceptional and are probably related to the hydrological conditions in this large lake, Meyen (1944) suggested that in species with long drawn-out breeding seasons divisions into " early " and " late " spawning groups might initiate speciation. Can this have happened in the case of *T. saka* and *T. squamipinnis* in Nyasa ? In Nyasa differences in hydrological conditions prevailing at different times of year may have been responsible for changes in spawning places following changes in spawning season, or conversely for changes in season following changes in place ; the evidence is not yet sufficient to say which is most likely. Frost (1956) has found that in the Windermere Char, *Salvelinus willughbii* Günther, differences in spawning season and the depth at which two populations spawn appear to be contributing to speciation.

The very restricted breeding seasons in Nyasa mean that relatively few batches of young are produced a year compared with most other *Tilapia*. Also, the Nyasa *Tilapia* have larger eggs and fewer eggs are laid at a time than in other *Tilapia* species (Lowe, 1955 c). In the Nyasa species the young are, however, brooded to a large size than in other species, which may lead to greater survival of the young. In Victoria *Tilapia* fry seem to have a critical stage and suffer a high mortality when about 15 mm. long, which is also the size at which these fry become independent ; in Nyasa *T. saka* fry are brooded until 24 mm. long, *T. squamipinnis* fry until 30 mm. long, and *T. lidole* fry until 52 mm. long, this may help them over a critical stage and appears to be an adaptation associated with more openwater conditions.

The possession of definite breeding seasons, and associated with these definite breeding places, means that waters away from the breeding grounds can be exploited at other times of year, the fish congregating at a well-defined and advertised rendezvous in the spawning season. This may have been an important factor in the colonisation of openwater in Nyasa by species which have to return inshore to spawn, such as *T. lidole* among the *Tilapia* and the zooplankton-feeding " Utaka " group of *Haplochromis*, some species of which return to the same areas to spawn for a month or six weeks every year (Lowe, 1952). (Indeed, the absence of definite seasons and places for breeding may be one of the reasons why Lake Victoria has not developed a comparable group of openwater zooplankton-feeding *Haplochromis*, as without such a rendezvous for spawning it is difficult to see how species occupying this openwater niche could evolve).

Thus to sum up, in Victoria, which is a relatively shallow lake, conditions are more uniform and the *Tilapia* move from zone to zone as they grow and make long movements about the lake, so there is no ecological or geographical

isolation ; neither is there any temporal isolation as the *Tilapia* breed throughout the year. Nyasa, on the other hand, is a deep lake so conditions are more varied ; although the Nyasa *Tilapia* are adapted for more openwater-living, water more than fifty metres deep presents an infrequently crossed barrier ; there is no direct evidence that the movements of the fish about this lake are as long as those in Victoria, but the brooding female makes quite long movements to inshore areas with family broods of young ; the presence of definite breeding seasons means that temporal as well as spatial isolation may have helped to keep new populations apart.

4. *Possible lines of evolution within Lake Nyasa*

The salient facts bearing on the relationships of members of the Nyasa *Tilapia* species complex appear to be : (1) *T. saka* and *T. squamipinnis* are morphologically most alike and appear to be the most recently separated species, (2) *T. lidole* is a specialised form for more openwater conditions which could most easily have arisen from a *T. saka*-like form, (3) The main mystery remains in the north, and in particular the relationship between *T. saka* and *T. karongae*. Very little is yet known about the biology of the *Tilapia* at the north end of the lake. *T. karongae* may be restricted to the northern end as this is the only part of the lake with permanent inflowing rivers ; this species appears to have a protracted breeding season, as is more usual for *Tilapia* at this latitude, but further work may show that all the species have a more protracted breeding season at the north end of the lake (which is, of course, nearer the equator) than they do further south.

Geological evidence suggests that the north is the oldest part of the lake (Dixey, 1941) and it would appear to be the cradle of the species flock, the ancestral *Tilapia*, thought to belong to the *T. galilaea* group of the genus (Trewavas, 1947 b), gaining access from the rivers to the new and deepening lake. This *Tilapia* would probably have had rather estuarine and inshore-dwelling habits at first, the young were probably not brooded to a large size, there would be a protracted breeding season as among *Tilapia* in the rivers of Rhodesia, and a dark, probably not very black, breeding male. As the lake deepened and with fluctuations in lake level this form may have become split into populations, becoming *T. karongae*, possibly in the estuary of one of the permament rivers, and a *T. saka*-like form, possibly in the main lake. The enlarged pharyngeal bones of *T. karongae* may have developed gradually in association with the silt burden of the river estuaries as suggested by Trewavas (1941), a suggestion supported by the enlarged pharyngeal bones noted in some *T. squamipinnis* from this area (Lowe, 1952, page 8).

The species *T. lidole* and *T. squamipinnis* would both appear to have evolved from a *T. saka*-like form after fluctuations and general increases in lake level brought more openwater conditions closer inshore ; deep water, only very infrequently crossed by the *Tilapia*, probably helped to isolate the populations of the incipient *Tilapia* species. The well-defined breeding seasons probably developed as the lake and *Tilapia* populations extended southwards and as the lake increased in depth and developed a regular hydrological cycle.

T. lidole shows greater morphological differences from *T. saka* than does *T. squamipinnis* which suggests that *T. lidole* originated earlier from a *T. saka*-like form, though many of the differences are adaptations associated with the more openwater habit of *T. lidole*. The possession of more or less the same breeding season in *T. lidole* and *T. saka* at the south end of the lake (Lowe, 1953) might suggest that the split came after the restriction of the breeding season, but this may be a parallel development in the two species, as *T. squamipinnis*, believed on morphological grounds to have evolved more recently than *T. lidole*, may have split off from a *T. saka*-like form before this restriction of breeding season. *T. lidole* retained the dark male breeding colour similar to that of *T. saka* and *T. karongae*. It is possible that the movements of the brooding females of the incipient *T. lidole* may have had a vital role in the evolution of this species (see page 10).

At another time and place, probably after some rise in lake level and perhaps further south where and when hydrological conditions facilitated the development of breeding seasons, the *T. saka*-like population appears to have divided, those fish breeding before the rains in very shallow water and retaining the dark breeding male becoming *T. saka*, and those breeding later, during the rains, in deeper water and developing a blue and white male becoming *T. squamipinnis*. Whether changes in breeding season or place came first is not known. Hydrological differences such as wave action might account for the later-breeding *T. squamipinnis* breeding in somewhat deeper water ; alternatively changes in depth of breeding places due to changes in lake level might, particularly if light is a controlling factor in determining spawning frequency as suggested by Aronson (1957), lead to later spawning in the deeper spawning populations (though this does not appear to have happened in the case of the deeper-spawning *T. lidole*). How much geographical barriers within the lake, such as barriers of deep water, aided this split is not known, and this remains the crux of this sympatric speciation controversy. The most probable sequence of events would seem to be that the lake rose and those fish which could spawn in deeper water stayed in these deepened areas and gradually delayed their spawning, then in some population semi-isolated by barriers of deep water a mutation occurred leading to a change in male breeding colour, after which selective breeding between fish with this colour and the later and deeper spawning habit enabled this population to keep distinct when the geographical isolating barriers were no longer effective. Such barriers would become less effective as the numbers of fish increased.

Throughout the ages fluctuations in lake level have probably helped to keep the different populations of the Nyasa *Tilapia* complex in balance, the more openwater-living forms increasing during periods of high lake level and the inshore-dwelling forms becoming relatively abundant in years of low water.

IV. SUMMARY OF CONCLUSIONS

1. Within the genus *Tilapia* three distinct types of breeding behaviour occur, the fish in the three groups being designated substratum-spawners, paternal-brooders and maternal-brooders. Structural and ecological differences are associated with behaviour differences between these three groups.

2. Substratum-spawners comprise only four known species, which are of wide distribution in Africa ; paternal-brooders are also few in number and appear to be confined to West Africa and the Congo. The numerous *Tilapia* species found in the waters of East and Central Africa, including all the species endemic to the Great Lakes, are maternal-brooders.

3. Breeding behaviour and ecological differences between the three groups are summarised.

4. The significance of these differences for evolution within the genus is discussed. Despite the probably greater antiquity of substratum-spawners, it is only among maternal-brooders that extensive speciation has occurred. Characters which may have accounted for the extensive speciation of the maternal-brooders include (a) preadaptation for more openwater conditions, both by ability to feed on phytoplankton and by brooding the eggs and young, and (b) the polygamous habit combined with the congregation of males on certain spawning grounds ; this may have had effects in the spread of mutations and also presents ideal conditions for sexual selection to operate and has probably contributed to the development of the striking male breeding colours. These breeding-colours advertise the spawning grounds so there is a well-marked rendezvous for spawning ; this effect is particularly noticeable in Lake Nyasa where there are well-defined breeding seasons, and permits the fish to exploit feeding grounds away from the spawning grounds at other times of year. The ability of the male mouth-brooder to fertilize continuously over long periods allows rapid spawning as pre-spawning courtship is reduced to a minimum.

5. Fish of the family Cichlidae speciate readily ; in the substratum-spawners speciation may have been inhibited by their restriction, both by food and spawning habits, to inshore waters where plants can grow ; thus populations can only spread contiguously along a shore line.

6. The uniform predominently vegetarian diet, found in all species of *Tilapia*, and the *Tilapia's* habit of moving from zone to zone as the fish grow (in contrast with the *Haplochromis* species which tend to remain in one particular ecological zone throughout life), and their long movements about a lake, would appear to have militated against the formation of *Tilapia* species flocks comparable with those of *Haplochromis* in Victoria and other lakes.

7. Nyasa appears unique in having a *Tilapia* species flock, evolved within the lake ; Lakes Tanganyika, Victoria and Jipe all have two endemic species of *Tilapia*, but in Tanganyika there is geographical as well as ecological isolation between the two species, and these may also have come from different stocks ; in Victoria the two species appear to have come from different stocks of *Tilapia*, and in Jipe the two species appear to have arisen by invasions at different times from the Pangani River.

8. In Nyasa the colonisation of new areas was probably promoted by the ability of the phytoplankton-feeding *Tilapia* to cross openwater combined with the necessity for them to move inshore to find suitable bottom for spawning ; the movements of the brooding females may have played a special part. In Lake Nyasa incomplete geographical isolation, brought about mainly by lake level changes and barriers of infrequently crossed water more than fifty metres deep, may have initiated speciation, but this has been supplemented

and superseded by ecological isolation, the *Tilapia* species separating to spawn at different depths, and temporal isolation, the species having well-defined breeding seasons which differ in the two most closely similar species, *T. saka* and *T. squamipinnis*, and a difference in colour of the breeding male between these two species. *T. saka* appears to be nearest the basal stock from which *T. karongae*, *T. lidole* and *T. squamipinnis* have been derived.

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SWIMMING AND FEEDING IN CRUSTACEAN LARVAE: THE NAUPLIUS LARVA

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(With 5 figures in the text)

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INTRODUCTION

There has been very little systematic research on the swimming, and still less on the feeding, of crustacean larvae. At the same time a proper understanding of the adult structures and functions and of the evolution of the group as a whole requires information which can only be obtained from study of the structure and functioning of the larva. The basic larval type in the Crustacea is the nauplius and its structure and mode of action form a natural starting point for such a study. Apart from early observations reported by Claus (1858), there was little but passing reference to swimming and feeding until 1924, when Cannon gave a brief account of the swimming and feeding of an estherid (Conchostraca) nauplius. He confirmed the observations made and views expressed in an account of swimming and feeding of *Chirocephalus* (Anostraca) (Cannon, 1928). In 1928 Storch published a much more extensive account of swimming and feeding in three types of copepod nauplii, *Diaptomus* (Calanoida), *Cyclops* (Cyclopoida) and *Canthocamptus* (Harpacticoida). Finally Lochhead (1936) and Norris & Crisp (1953) have given accounts, which substantially agree, of feeding in nauplii of *Balanus* (Cirripedia). The specialised nauplii of many parasitic copepods are not considered in the following account.

MATERIAL AND METHODS

Observations and dissections have been made on nauplii of *Artemia* (Anostraca), *Balanus* (Cirripedia), *Calanus*, *Temora*, *Euchaeta*, *Acartia* and *Oithona* (Copepoda), *Meganyctiphanes*, and *Thysanoessa* (Euphausiacea).

The anatomy of the nauplii and the form of the appendages have been studied mainly by microscopic examination of intact living or freshly killed nauplii. Details of setae and their setulation were elucidated on isolated limbs but an isolated limb is usually flattened by a coverslip and natural forms cannot safely be inferred from examination of such preparations. Sections of embedded material have not been used but hand sections of fresh nauplii, particularly sagittal sections, were valuable in elucidating the relationships of the limbs one to another and to the other parts of the body ; these were made under a binocular microscope with a sharp scalpel.

The accounts of swimming are based mainly on observations made under a binocular stereo-microscope with magnifications from twenty to eighty times. Details of limb positions and movements occasionally require higher magnifications but higher magnifications require some sort of restraint of the nauplius' activity and were used sparingly. Much very valuable confirmation and elucidation of the direct observations was obtained by microphotographs taken by electronic flash, giving an effective exposure of about 1/5,000th of a second.

Unless it was necessary to restrict the movements of the nauplii, they were examined when swimming freely in cells about 1 mm. deep. When it was necessary to restrict movement, the nauplii were examined on an ordinary flat slide, or a cavity slide with a lenticular cavity, with a small quantity of water. From time to time, as the nauplius approaches the periphery of the drop, it comes to a standstill for a few seconds " swimming " vigorously. This happens most easily on a slightly waxed or greasy slide in which the angle of contact of the water is steep and this method of restricting movement is, in my opinion, preferable to the use of coverslips or gum solutions. If the drop on a slide spreads out into a shallow film the nauplii can often progress in water which is clearly too shallow for normal swimming and while limb movements are usually markedly slowed down in such circumstances they may also be distorted in other ways. Some observations were made with a stroboscope disc interrupting the light reaching the microscope mirror, but these were only occasionally fruitful : copepod nauplii swim too sporadically and too unevenly to be good objects for stroboscope examination.

THE NAUPLIUS OF *ARTEMIA*

Before describing the swimming and feeding movements it is necessary to give a brief account of the structure of the appendages and the other organs involved in a Stage 1 nauplius (Fig. 1 a).

Antennules. In *Artemia*, as in other branchipods, the antennules are very simple in structure ; they are unsegmented and bear only two terminal setae.

Antennae. The antennae comprise a sympod of two segments and an unsegmented simple exopod and endopod. The first segment bears at its distal end a stout curved spine. This spine is frequently referred to as a " masticatory process " but there is very little justification for the use of this term because nauplii do not in fact use these spines to masticate, i.e. break up, their food. The bifid point of this spine lies just behind the mouth, between

the long labrum and the ventral side of the body. About a quarter of its length at the pointed end is setulose, the setules apparently lying all round the process, rather like a bottle-brush. The second segment bears a single posterior seta at its distal end. This seta is long and tapering and its distal half has two rows of setules, one dorsal the other ventral, which slope slightly towards the animal's body. The exopod bears two terminal setae, both rather short but one stouter than the other, and a row of seven long ventral setae with no setules but with a distinct hinge at about their mid-point. The endopod is quite short, only one-third of the length of the exopod and bears only two terminal setae, of the same form and approximately the same length as the ventral setae of the exopod. These two terminal setae and the ventral

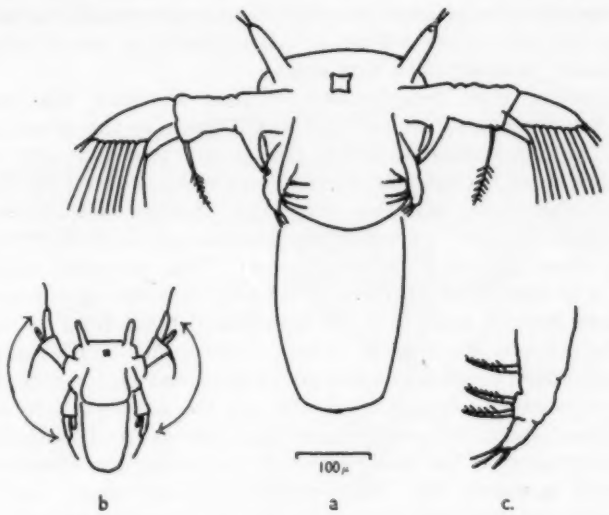

Fig. 1.—*Artemia* : 1st Nauplius. (*a*) ventral view to show form and setation of limbs. (*b*) diagram to show extent of antennal stroke. (*c*) mandible enlarged to show setation.

setae of the exopod, form a single functional series, which provide the main propulsive surface of the limb.

Mandible. The mandible is uniramous and approximately the same length as the sympod of the antenne. Its segmentation is not clearly marked but it appears to comprise a large basal part (the sympod) and a short terminal joint (the endopod ?). There are six setae ; a terminal group of three rather short, smooth setae and three stout, setulose spines on the inner side of the sympod. As the two mandibles work more or less in plane parallel to the ventral surface of the body, the inner spines of one mandible approximately oppose those of the other. The setules are regularly arranged in two ranks, and point nearly dorsally and ventrally with a slight slant forwards, i.e. towards the mouth.

Labrum. The labrum of the branchiopodan nauplius is very large. In

Artemia, when lying against the ventral surface, it overlaps the mandibles almost completely. In life it is very mobile and may be extended almost vertically downwards from the body.

The cephalic appendages and movements of the second stage nauplius are not significantly different from those of Stage I or the later stages ; even as late as the seventh instar, in which eight pairs of trunk limbs are functional swimming organs, the essential structure of the mouth parts is unchanged : at this stage, for instance, the mandible has five not three stout feathered setae on its inner edge but their structure and disposition are similar to the three of the second nauplius and obviously the two extra setae amplify the action of the three original ones without introducing any new function. Observations and comments on any stage of the life history in which the antennae are active propulsive limbs seem to apply equally to all of these stages. The account which follows is based mainly on second stage nauplii which are easier to study than first stage.

It is apparent from consideration of their structure that neither the antennules nor the mandibles can contribute much to the propulsion of the nauplius. The antennules are feeble and poorly provided with setae ; the mandibles are stout enough but almost completely enclosed by the labrum. Observation of a living nauplius completely confirms this inference. The antennules scarcely move as the animal swims and such slight movements as they do show are plainly a consequence of the powerful strokes of the antennae. The mandibles move quite actively but the arc of movement is restricted and through most of it the mandible is overlapped by the labrum. These limitatons and the absence of long " swimming " setae make it quite clear that very little propulsive force can be produced by the mandibles. The sole effective swimming organs, therefore, are the antennae. In addition to their movement about the origin of the limb, the sympod is flexible so that the arc of movement of the distal segments and setae, the effective propellor of the animal, is nearly 180°. The stroke is a very simple one : the back stroke is made with the limbs fully extended and the setae spread, so that the limb has the largest possible effective area ; in the forward recovery stroke the limb is flexed and the setae trail, so that resistance is reduced to a minimum. The two antennae normally beat in unison and turning movements appear to be executed by varying the speed of the strokes of the two antennae. The mechanism for extending and flexing the limbs during the swimming stroke is partly an active and partly a passive one : the extending and flexing of the axis of the limb is very largely, but probably not wholly, under muscular control : the spread of the seta is largely, if not wholly, a passive process, controlled by the resistance of the water. This is most obviously true of the bending of the long setae at their hinges.

The antennules take no part in the feeding mechanism, and their function is mainly sensory, perhaps mainly concerned with balance. But both the antennae and the mandibles play a part in feeding, the antennae principally in gathering the food, the mandible in transferring it to the mouth. The swimming stroke of the antennae ends with the long ventral seta sweeping inwards, round the posterior edge of the labrum so that any particles

encountered by these setae during the stroke will be carried into the "vestibule" formed between the posterio-dorsal surface of the labrum and the ventral surface of the body (the "atrium oris" of Eriksson, 1934). The movements of the mandible are usually restricted to a comparatively small arc of 30–40°. The outer limit is readily observed and at it the limb lies at an angle of not more than 30° to the longitudinal axis of the body ; the inner limit is exceedingly difficult to observe and while the basal part of the limb stops when it is approximately parallel to the body its apex appears to be flexed rather further. A stroke through an arc so limited is almost exactly the stroke needed to comb particles from the long setae of the mandibles, and the terminal setae of the mandible appear to comb the antennal setae in just this way. Two opposite movements contribute to this combing : as the antennae begin their recovery stroke, the long setae are drawn outwards and

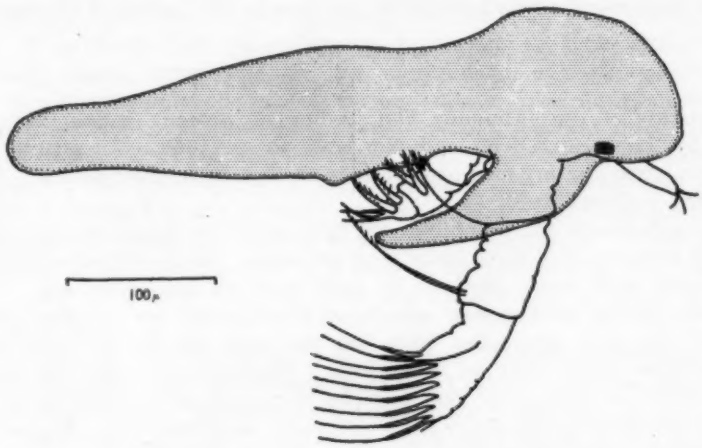

Fig. 2.—*Artemia* : 2nd Nauplius. Sagittal section.

forwards, the mandibles are completing their inward stroke and their terminal setae are moving inwards. Whether this combing action is continuous and automatic in swimming or not, it is obvious that the three terminal setae of the mandible cannot effectively clean the nine antennal seta in a single stroke and it is easily observed that, in rather thick suspensions of particles, from time to time the antennal setae are drawn up to the mandibles and "groomed" more elaborately. It is possible that the stout feathered setae of the mandible assist in this combing.

The further activities of the mandibles are obscured by the overhanging labrum and have to be inferred from the structural relations of the parts, which can best be understood by reference to a sagittal section of the larva. Here it can be seen that the axis of the mandible lies parallel to the ventral surface behind the labrum ; the three setulose spines lie approximately horizontally, pointing slightly downwards and forwards, and the setules on

these setae lie approximately normal to the ventral surface, but all pointing slightly forwards towards the mouth. Secondly, examination of the mandible itself shows that, in addition to the muscles which produce the main oscillation of the limb, there are two smaller muscles inserted in the small terminal segment (Claus 1886, pl. VIII, Fig. 1) whose function must be to flex the distal part of the limb, and so to swing forward the tips of the three mandibular spines. It may be inferred then that, as the mandibles complete their stroke they are flexed so that these three spines swing forwards carrying food particles in the vestibule forwards to the mouth where the basal spines of the antennae push them into the mouth.

Cannon (1924, 1928) lays great emphasis on the presence of a sticky secretion of the labral glands, to entangle the particles so that they can be pushed into the mouth. While the presence of such a secretion would make the capture and manipulation of small particles easier, I have never been able to demonstrate convincingly its presence in the region of the mouth.

THE NAUPLIUS OF *BALANUS*

Accounts of the swimming and feeding of *Balanus* nauplii have been given by Lochhead (1936) and Norris & Crisp (1953). In general these accounts agree and my own observations confirm their observations. A brief summary is given here.

The antennules and mandibles are both relatively larger than in *Artemia* and the setation of all three limbs more extensive (see Lochhead's Fig.). The antennules seem most often to be held more or less motionless pointing forwards but are used at least sometimes as swimming limbs. The antennae are still the main propulsive limbs but the large mandibules must make a considerable contribution to locomotion. Similarly, although the antennae assist in the sweeping in of food particles, the principal food collecting organs are the mandibules, especially their endopods, with their long plumose setae. In addition there are setulose spines on the ventral side of the mandibles which, with the jaw process of the antennae, are concerned with the transport of the food to the mouth. Both Lochhead and Norris & Crisp state that the mouth of *Balanus* nauplii is very narrow and only particles less than 6–8 μ in diameter are ingested. Minute particles are collected and carried towards the mouth partly by the setae of the mandible and, to some extent, of the antennae, but partly also by the suction of the forward stroke of the antennae. Both accounts draw attention to the denticulate areas on the ventral surface of the body behind the mouth especially on the abdominal process : Lochhead believes that their chief function is to prevent escape of particles from the " oral groove " but Norris & Crisp suggest that they are used to clean particles off the mandibular setae and possibly to break up larger particles, such as the small algal filaments which both they and Lochhead show to form part of the food of the nauplii. Norris & Crisp draw attention to the fact that the cirri of the adult develop from the abdominal process and that these movements of the abdominal process of the nauplius foreshadow the feeding movements of the cirri in the adult.

NAUPLII OF CALANOID COPEPODS

Storch (1928) has described the swimming and feeding of *Diaptomus* nauplii but my observations differ markedly from his and discussion of his account is therefore postponed.

Most of the observations and dissections have been done with the third nauplius of *Calanus finmarchicus*, the most readily obtained large copepod nauplius. Eggs of *Calanus* are readily obtained by isolating ripe females in small crystallizing dishes, and hatch in about twenty-four hours. The first two nauplii do not feed (Marshall & Orr 1956) but the third nauplius feeds actively and proved excellent material. Later stages of *Calanus* and various stages of *Temora* and *Acartia* were picked out of fine tow-nettings and studied as they became available.

No significant differences were found between these species and, while the setation of the limbs of the later stages is more elaborate than in the earlier stages, there appears to be no changes in the manner of function of the limbs. Thus though N VI has maxillulae approaching the form of those of the copepodite stages, these maxillulae, like the rudiments of the maxillae behind them, lie closely against the ventral surface and are quite functionless. The manner of swimming and feeding appears to remain constant throughout the nauplius stages. There is one development which may have some functional significance ; the basal endite of the mandible, which is a rounded lobe in nauplius 3 bearing one of the setulose spines elongates and in stages 5 and 6 is a long curved process, terminated in a group of small teeth. It still bears a setulose spine and can function as it does in the earlier stages but it could also be used, as it is in the copepodite and adult stages, to break up large particles of food.

One other species of calanoid was examined, *Euchaeta norvegica*, which differed markedly from the species mentioned above. An account of the naupliar stages of this species is given by Nicholls (1934) who showed that it did not feed, and has none of the setae which were found to be principally concerned with feeding in the other species.

The structure and setation of the limbs of *Calanus* nauplii have been described by Lebour (1916) and Sømme (1934) and the information is summarised by Marshall & Orr (1955). But none of these authors were particularly concerned with feeding, so that a description of the limbs, drawing attention to points of special interest is desirable before considering their functions. *Calanus* N III is taken as the type for discussion.

Antennules : The antennules are approximately as long as the antenna and distinctly flattened laterally, especially in the distal half. They are uniramous, with one distal segment clearly separated and a second adumbrated along the ventral edge, and there is a terminal group of four long setae and two long seta on the dorsal edge of the distal segment. None of the setae have setules.

Antennae (Fig. 3 b) : The antennae are biramous and, though the segmentation is not always clearly marked, the limb can be seen to comprise a sympod of two joints, an endopod, apparently of two joints and an exopod of seven

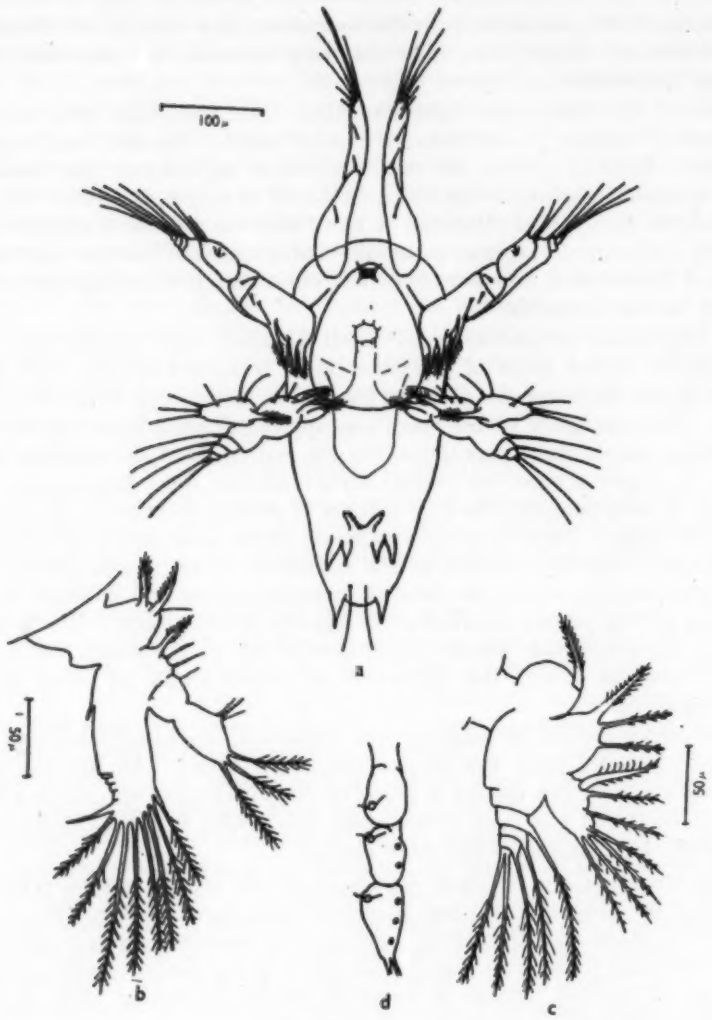

Fig. 3.—*Calanus*: 3rd Nauplius. (*a*) Ventral view to show resting position of limbs. (*b*) Antenna. (*c*) Mandible. (*d*) diagram showing the arrangement of the setules on mandibular endites.

segments (Marshall & Orr 1955). The setation is as follows :—

(i) Coxa — 2 stout, curved setulose spines, and 1 fine, smooth seta on the ventral side.

(ii) Basis — 1 stout, curved setulose spine, 2 fine, smooth setae on the ventral side and 1 small dorsal seta.

(iii) Endopod 1 straight seta on first joint, a group of 3 small seta on the second joint and a terminal group of 3 long, plumose setae.

(iv) Exopod a graded series of 8 plumose setae round the distal end and a short, stout subterminal seta on the dorsal side.

The three stout spines on the coxa and basis lie close to the labrum at the side of the mouth and are clearly concerned in pushing food to the mouth. Similarly, the eight plumose setae of the exopod and three terminal plumose setae of the endopod together form the main propulsive setae of the appendage.

Mandibles (Fig. 3 c) : The mandibles, like the antennae are biramous about two-thirds of the length of the antennae but considerably broader and distincly flattened. The sympod comprises two joints both of which have broad, rounded endites and form together a flat, leaf-like limb.

The endopod is unsegmented but the exopod has five distinct segments. The setation is as follows :—

(i) Coxa a single setulose spine.

(ii) Basis a setulose spine and 2 slender, plumose setae.

(iii) Endopod a stout setulose spine near the base, 3 ventral plumose setae and a terminal group of 4 plumose setae.

(iv) Exopod a distal fan of 5 plumose setae.

It can be seen that, like the antennae, the mandibles bear three stout, setulose spines and a distal fan of long plumose setae, which are clearly propulsive, whether or not they are concerned in food collecting as well. But, unlike the antennae on which all the other ventral setae are short and smooth, the mandible has a series of long plumose setae along its ventral edge.

Labrum : The labrum is distinctly shorter than in *Artemia* and in profile it is bluntly triangular. The mouth faces backwards, close to the base of the labrum, and is scarcely overlapped by it at all.

As Claus (1858) first showed, calanoid nauplii swim in two distinct ways—in a rather slow, smooth glide and in very rapid, often erratic, darts. This second darting movement is almost impossible to analyse because the animal disappears from the field of view of the microscope so rapidly. It can, however, be seen that all three pairs of appendages are involved and make strokes through considerable angles. Since there is a clear, metachronial rhythm in slower movements it is probable that the limbs beat metachronially in rapid movement too. The rate of movement of calanoid nauplii swimming in this way far surpasses the fastest swimming of *Artemia* and *Balanus* nauplii and may be correlated with the relatively much longer appendages and the fact that three pairs, not one as in *Artemia*, are involved.

In the slow gliding movement, the antennules are nearly motionless and extended straight forwards, in the usual resting position. The antennae and mandibles move with a rapid stroke, through a rather shorter arc than described above. There is a distinct difference of phase between the movements of the antennae and the mandibles. The limbs appear to be held more stiffly during the recovery stroke than in either *Artemia* or *Balanus*, although the setae are flexed passively by their resistance to the water. However considerable reduction of the effective area of the limbs is achieved by closing

the terminal fans of the setae during the recovery stroke. The terminal fan of setae of either of these limbs is spread or closed by small movements of the terminal joints controlled by the muscles of the limb, and the presence of a number of distinct joints close to the apex of the limb in copepod nauplii may be correlated with this manner of reducing resistance to the recovery stroke.

In his account of feeding in *Diaptomus* nauplii Storch (1928) describes four streams of water passing between the limbs to the mouth. I have never been able to detect such streams. The clearest information about the manner of collecting food was obtained from observations of nauplii in a suspension of the dinoflagellate, *Peridinium trochoideum*. This is a unicellular organism, with dimensions of the order of 25 μ, so that it is possible to follow the path of individual cells even at low magnification ; it does not aggregate in chains as diatoms do and it is freely eaten by calanoid nauplii. When a nauplius is swimming slowly with the vibratory movement of its antennae and mandibles, in such a suspension, the most obvious current is a wide, backward stream lateral and ventral to the animal. Viewed from in front it occupies a broadly U-shaped area, corresponding more or less closely to the area swept by the antennae. Particles, like cells of *Peridinium* entering this stream are swept rapidly backwards and give no sign of any inward vortices such as Storch describes. Viewed laterally, the stream passes backwards and downwards away from the body and there is no evidence of " backwash " behind the labrum, as Cannon (1924, 1928) described in *Estheria* and *Chirocephalus*. Indeed, since in calanoid nauplii the posterior part of the body is held nearly vertically downwards, it is difficult to see how an effective backwash could develop. There is thus no evidence of particles being carried to the mouth by water currents. It is moreover apparent that only those peridinians which come within reach of the mandibular setae are ultimately ingested.

At its extreme forward position the axis of the mandible lies at an angle of 80° to the long axis of the body. The limb, with its setae, forms a curved surface, concave behind, and at the beginning of the backstroke, the setae on the ventral edge are slightly in front of the axis of the limb (which is near its dorsal edge) and the concave surface faces back and slightly downwards. During the stroke as the limb moves radially round its point of attachment, it rotates on its own axis until at the end of the stroke, when the axes of the two mandibles are parallel to each other and to the long axis of the body, the blades of the limbs are lying in a plane approximately parallel to the ventral surface of the body with their concave posterior surfaces upwards facing the ventral surface of the body. In this position the setae of the two mandibles meet in the middle line to form a ventral basket, behind the mouth. Thus when a particle enters the area swept by the mandibles it is carried backwards, but also inwards and upwards and is enclosed in this postoral basket. A particle so gathered in by the mandibles can usually be seen passing through the oesophagus (which happens to be in approximately the same focal place as the mandible at its final position) and into the stomach, so that there can be no doubt that such particles are ingested. It remains to be shown how a particle captured in this way is passed to the mouth, a process which is very rapid.

Examination of a sagittal section of a nauplius (Fig. 4) shows that the setae which form the basket are primarily the five slender plumose setae of the ventral edge of the mandibles. The three stout setulose spines lie closer to the body than the setae which form the basket and point more or less towards the mouth. In addition, the three stout setulose spines of the antenna lie in the anterior part of this region just behind the labrum. It seems reasonable to assume that the mandible flexes slightly as it completes its stroke and so the particles enclosed by the setal basket are swept forwards by the three mandibular spines to the region immediately behind the mouth. Here the spines of the antennae, which complete their stroke shortly after the mandibles, push the particles into the mouth.

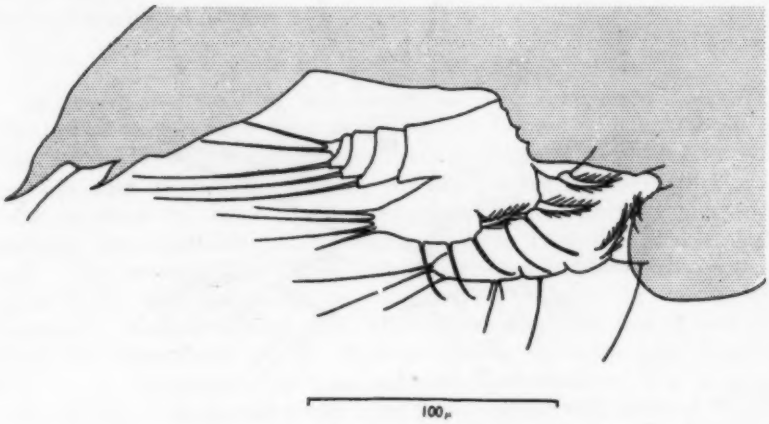

Fig. 4.—*Calanus* : 3rd Nauplius. Sagittal section.

The spacing of the setae which form the post-oral " basket " and so actually collect these particles, varies from about 15 μ to 20 μ so that this feeding mechanism is obviously adapted to deal with relatively large particles. If smaller particles were present in the water, some of them would be carried in on the setae, which are setulose, but collection of such particles would be inefficient. The setules on the antennal and mandibular spines are about 3 μ apart (Marshall & Orr 1956) and so are capable of carrying to the mouth quite small particles. It is possible that small particles (5–10 μ say) would be rather inefficiently collected by the mandibular setae and would be transported to the mouth by the setulose jaw-hooks. But it is difficult to suggest a mechanism of true filter feeding in the nauplius, which can be compared with the filter feeding mechanism of the adult.

THE NAUPLII OF CYCLOPOID COPEPODS

Nauplii of *Oithona similis* were abundant in the plankton at Millport and a number of observations were made on its structure and movements. Storch (1928) gave an account of the swimming of the nauplius of *Cyclops strennus* :

my observations agree very closely with those of Storch and only a brief account is given here.

The structure and setation of the limbs of the nauplius of *Oithona* are described and figured by Oberg (1906) and differ only in minor details from Storch's account of *Cyclops*. The antennules are of three segments : the second segment has two short ventral setae and a long distal setae, which with the three distal setae of the third segment provide the propulsive fan of the limb. The antennae are biramous with a two segmented sympod, an endopod of one segment and an exopod of five segments. The coxa has two stout setulose spines ; the basis has two or three short ventral setae and the

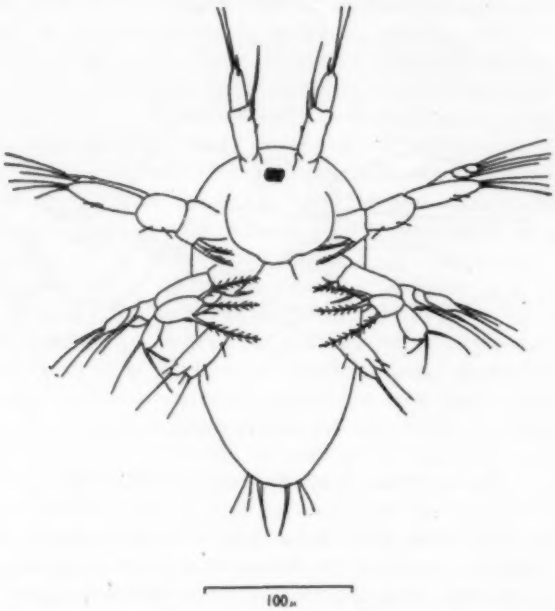

Fig. 5.—*Oithona* : 4th Nauplius. Ventral view.

endopod and exopod bear a fan of distal setae which are plainly propulsive in function : the antennae therefore are very similar to those of the calanoid nauplii. The mandibles are again biramous with a sympod of two segments, and endopod of two segments and an exopod of four segments. The coxa and basis are both short and bear respectively one and two rather stout plumose setae. The first joint of the endopod bears a triangular plate (endite) with three or four long plumose setae, which lie horizontally pointing more or less towards the middle line. The second segment of the endopod bears three terminal setae and the exopod five distal setae which are apparently primarily propulsive. The maxillulae appear earlier in the cyclopoids than in calanoids and in *Oithona* N VI are quite large, movable limbs but they do not appear to be important functionally.

The resting position (Fig. 4) and stroke of the limbs appear to be much the same as in calanoid nauplii but cyclopoid nauplii never swim slowly with the vibratory movement of antennae and mandibles : the swimming is always rather rapid and jerky, though not in vigorous bounds like the rapid movement of calanoid nauplii, and the antennules appear always to be beating with the other two pairs of appendages. There is as usual a metachronal rhythm.

The principal feeding organs are the mandibles and, particularly, the plumose setae of the triangular endite which work as a kind of sweep net to gather particles from the water and concentrates them in the post oral region : here they are pushed forward by the coxal and basal setae of the mandible and then into the mouth by the setulose spines of the antennae. The feeding mechanism in the *Oithona* and *Cyclops* is therefore essentially the same as that of the calanoid nauplii.

NAUPLII OF PENAEIDEA AND EUPHAUSIACEA

Preserved specimens of the nauplii of *Meganyctiphanes norvegica* and *Thysanoessa raschii* have been examined but I have been unable to obtain living material. Sars (1898) gives an account of the larvae of *Meganyctiphanes norvegica* and *Thysanoessa raschii*, without clearly differentiating the two forms, in which he states that the nauplius swims mainly by means of the antennae and mandibles, the antennules being generally held straight in front. The metanauplius (third stage) has its mandibles reduced to small conical buds, and locomotion is " exclusively effected by means of the antennae ". Lebour (1924, 1925, 1926) gives accounts and figures of the nauplii of *Nyctiphanes couchi*, *Meganyctiphanes norvegica* and *Thysanoessa inermis*. In *Nyctiphanes* the eggs and nauplii are retained in a brood-pouch until just before the second moult : the metanauplius, like those of *Meganyctiphanes* and *Thysanoessa*, has reduced mandibles and it does not feed (Lebour 1924). Lebour says nothing of feeding in the other two species but Macdonald (1927) states that the nauplius of *Meganyctiphanes* has no mouth, and that although the mouth is open, the metanauplius does not feed.

From morphological examination of the appendages it is quite clear that these euphausiid nauplii cannot feed. Neither the antennae nor the mandibles have any ventral setae, corresponding to the setae used in feeding by copepod or cirripede nauplii ; in the third stage (metanauplius) the mandibles are reduced to a blunt rudiment, through which can be seen the functional jaw of the next larva, the first calyptopis. On the other hand the distal setae of all three pairs of limbs, the setae concerned most with swimming, are quite long and are all setulose.

Heldt (1938) gives a very full account of the nauplii of three species of Penaeidea which occur in the Mediterranean. Their appendages closely resemble those of euphausiid nauplii except that the mandibles are never reduced as they are in the third nauplius of Euphausiida, and in the last stages have a distinct basal swelling which foreshadows the corpus mandibulae of the protozoea but has no function in the nauplius. Throughout all the naupliar stages, young penaeids do not feed but live on masses of stored yolk ; the alimentary canal is fully formed only in the last nauplius stage. She gives a

brief account of swimming of the nauplii, in which she states that nauplii swim with all three pairs of appendages which they use as oars. Swimming is intermittent, a dozen or so quite rapid strokes, lifting the nauplius upwards, are followed by a resting period, in which the nauplius falls gently back to approximately to the point at which it started. This account calls to mind the manner of " hop and sink " swimming of (adult) *Calanus*, (Bainbridge 1952). Heldt suggests that penaeid nauplii do little more than keep themselves off the bottom by swimming and points out that she was unable to find nauplii in tow-nettings until she attached her nets to the frame of a trawl. Euphausiid nauplii are usually found near the surface but euphausiid eggs are not demersal like those of Penaeidea and the nauplii may have very similar swimming habits.

DISCUSSION

1. *Swimming*

The swimming mechanism of nauplius larvae appears to be uniform and very straight forward. Propulsion is produced by a simple paddling stroke of the appendages, which are rigidly extended on the backward, driving stroke and flexed, partly passively and partly by active muscular control, on the forward stroke. Variation occurs in two points, in the number of pairs of appendages involved, and in the mechanism of flexing the limb. The number of limbs most often used is two. The antennules appear to be mainly balancing organs but are used as locomotor organs in the rapid leaps with which a copepod nauplius escapes from a disturbing stimulus and at times by cirripede nauplii and possibly other nauplii in similar circumstances. The antennae are always large and in nearly all cases are mainly, if not wholly, locomotor in function. The principal exception is *Artemia* in which it is also the chief food-collecting organ. In a few cases as in *Artemia* and the third nauplius (metanauplius) of euphausiids, the mandibles are reduced and in *Artemia* are only concerned in feeding : in the euphausiids which have been studied (Sars, 1898 ; Lebour, 1924 ; Macdonald, 1927) the nauplius does not feed and the mandibles in stage 3 are reduced to functionless buds. In most other nauplii the mandible is large and even where its structure is plainly adapted for food gathering it must contribute significantly to locomotion.

Reduction of resistance to forward movement in the recovery stroke is achieved in part by flexing the distal part of the limb but mainly by reducing the resistance of the setae to the water. This is done in two ways. In *Artemia* the main swimming setae are hinged and flexed passively on this hinge during the recovery stroke. In copepod and cirripede nauplii and in euphausid and penaeid nauplii the setae are not hinged and variation in the effective area of the setal paddle is brought about by flexing and extending the apical segments of the exopodite of the limb so that the setae, which are set on these segments, are opened and closed like a fan.

Although the mechanism of swimming is uniform the swimming habits of different nauplii are varied. Branchiopod and cirripede swim with relatively

slow strokes and, compared with copepod nauplii, their swimming appears laboured and inefficient. From the appearance of their appendages it seems likely that euphausid and penaeid nauplii would give a similar impression. Copepod nauplii give a much more businesslike impression : the strokes are more rapid and progress less jerky. In the Cyclopoida, progress is always fairly rapid and the antennules appear to be involved in propulsion, although this point was not quite clear ; the Calanoida, on the other hand, have two modes of swimming. The first is a smooth, relatively slow, gliding motion, produced by rapid, vibratory movements of the antennae and mandibles only ; this type of swimming is normally accompanied by the collection of food particles. The second is a rapid bounding movement, produced by powerful strokes of all three pairs of appendages, and is used in escaping capture, or avoidance of an unpleasant stimulation.

2. *Feeding*

The feeding mechanism of nauplius larvae is a more complex matter. In the first place, my own observations show that there is a divergence between the nauplii of the Branchiopoda as exemplified by *Artemia* on the one hand and other nauplii on the other. And secondly there is a divergence between my account above and other published accounts of feeding in both these groups. Thirdly there is a number of nauplii, in various groups, which do not feed. There can be little doubt that the nauplii which do not feed are specialised in this respect and that the absence of a functional mouth and feeding appendages is a secondary condition.

The difference between the branchiopod nauplius and other nauplii lies in the relative importance of the antennae and mandibles as food collecting organs. In the branchiopod nauplius, the antennae are the principal food collecting organs and the mandibles are always relatively small, while in the other nauplii the antennae are almost entirely swimming organs, with only a minor role in feeding. Cannon (1928) describes the feeding mechanism of *Chirocephalus* thus :

" The heavy armature of setae on the antennae and mandibles not only makes them efficient swimming organs, but converts them at the same time into a sort of casting net. During the swimming stroke all the natatory limbs converge on the hinder part of the body and, in so doing, sweep back on to the body any floating particles with which they have come in contact. Then as the limbs relax and the body moves forwards as a result of the swimming stroke, the suction under the labrum sucks the particles into the mouth region ". (p. 814). It is not made clear in this account to what extent each of the two pairs of natatory limbs contribute to the food collection but it is clear from any of the published figures of branchiopod nauplii (Claus 1886, Pl. II, Fig. 1 or Cannon & Manton 1927, text-fig. 14) that the mandibles are relatively short and the antennae must provide the principal part of the sweep net. On the other hand the published figures, e.g. those of Heath (1924), as well as my own observation show that the mandibles of an *Artemia* nauplius are relatively shorter than those of the other genera and it is possible

that in these other forms the mandibles play a larger part in food collecting, than they do in *Artemia*.

The principal difference between my account of the feeding of *Artemia* nauplii, and Cannon's account of that of *Estheria* and *Chirocephalus* lies in our interpretation of what happens to the particles collected by the antennae. According to Cannon (1924 p. 398, 1928 p. 814) the particles are concentrated in the mouth region by the suction produced behind the large labrum as the nauplius swims through the water and then entangled in the sticky secretions of labral glands. The ball of secretion and particles so formed is then pushed into the mouth by the basal jaw processes of the antennae.

This account can be criticised on several points. Firstly, the animal moves forward most rapidly during the propulsive stroke of the limbs, which must produce a positive pressure behind them, and slows down distinctly when the limbs relax, reducing any possible negative pressure behind the labrum. In the second place, suction due to forward movement of the body can hardly be greater than that produced by the forward movement of the limbs. Thirdly this account assigns no function to the setae of the mandible which are stout and setulose and quite clearly have some function. Fourthly it seems to imply that the particles are passively entangled in the sticky secretions of the labral glands while it is very obvious that the mandibles are actively engaged in some kind of manipulative or masticatory movement, whenever the animal is swimming and so, potentially at least, feeding.

While suction, chiefly produced by the forward movement of the antennae, no doubt carries smaller particles forward into the vestibule behind the mouth, it seems much more likely that food particles are carried to the immediate vicinity of the mouth mainly by the setae of the mandible, and they are finally pushed into the mouth by the basal spines of the antennae. The importance of labral gland secretions in this process is not clear. I have never been able to demonstrate their presence convincingly and, while the presence of such secretions would make the capture and manipulation of small particles easier, sticky secretions do not seem to be a necessary part of the food collecting apparatus.

The accounts given by Lochhead (1936) and Norris & Crisp (1951) for barnacle nauplii and that given above for copepod nauplii all agree in describing the mechanism of food collection as a sort of double " sweep net ", formed by the mandibles. Particles in the water swept by the mandibular setae are carried inwards to the middle line by the long ventral setae of the mandibles and, as the mandibles flex slightly at the end of the stroke, are pushed forward to the mouth by the setulose spines of the proximal end ces. The forward movement of particles within the " basket ", formed when the ventral setae of the two mandibles meet in the mid ventral line, is almost certainly assisted by passage of water forwards as the broad blade of the limb closes against the ventral surface of the body : escape of water backwards is prevented by the downward flexure of the abdomen. Finally the particles enter the mouth, pushed by the basal setae of the antennae, as they complete their swimming stroke, and sucked by muscular expansion of the oesophagus. The rapidity with which particles enter the oesophagus after capture suggests that only a

single stroke of the limbs is necessary to complete the capture and ingestion of a particle.

As indicated above, in *Balanus* nauplii only very small particles are ingested and the suction produced by the forward stroke of the antennae may be important in the transport of these particles. In addition, the cirripede nauplii have in the spines of the abdominal process, a mechanism for triturating algal filaments and similar material collected by the mandibular setae but too large for ingestion. The use of the abdominal process appears to foreshadow the adult feeding mechanism, since the cirri of the adult are developed from this region. There are tracts of setules and spines on the ventral side of copepod nauplii, as Storch (1928) points out, but they are restricted to the immediate post oral region, and though they may assist in preventing the escape of particles collected by the mandibles, I have never seen them used, as they are in *Balanus*, for triturating large particles. The large thorn like processes on the abdomen of *Calanus* nauplii appear to have no function connected with feeding.

The manner of feeding is broadly the same in both calanoid and cyclopoid nauplii, and follows quite closely the account of the feeding of *Cyclops* nauplii given by Storch (1928) but differs profoundly from the account of the feeding of *Diaptomus* nauplii given in the same paper. Storch begins his account of *Diaptomus* with a description of the limbs, of which the relevant parts are his statements that the antennae and the mandibles are constructed in essentially the same way differing only in size, that both bear three or four (depending on the stage) stout, feathered setae (Kauhaken) on the basal endites, and that the setules of these setae, on both appendages, meet across the spaces between the setae and form a close filter of backwardly pointing setules. During the slow swimming of the nauplius, these two pairs of appendages vibrate rapidly slightly out of phase, and pump water forwards in two streams on each side, one between the antennae and mandible and the other between the mandible and the ventrally projecting " lower lip ". Particles are strained out of these water streams and pushed towards the mouth where they are sucked into the mouth by muscular expansion of the oesophagus.

There can be no doubt that this account is wrong in several important respects. The antennae and mandibles can be said to be constructed in the same way only in the broadest sense, and, are markedly different in those parts relevant to the feeding mechanism. Both pairs of limbs do bear three or four stout, feathered setae, the setulose spines of the account above, but the arrangement of these setae differs markedly : in the antennae they are concentrated at the base of the limb and might close the gap between the bases of the antennae and mandibles but their setules are not all arranged in one plane and do not form a filter as Storch states ; on the mandibles the setulose spines are distributed rather widely along its ventral surface, so far apart that the spaces between them could not be closed by the setules, which in any case are mainly more or less normal to the plane in which the three spines lie (Fig. 2 c). These structural facts make it clear that the mandibular setulose spines at least cannot act as a filter such as Storch describes. While it is

almost certain that some water does pass out through the spaces between the bases of the antennae and mandibles it can readily be seen that the basal spines of the antennae do not in fact form a filter across this gap, but, at the end of the antennal stroke, pass into this gap, close to the posterior surface of the labrum and their function is quite obviously that of pushing food particles brought to the immediate post-oral region into the mouth. In any case the volume of water which could be so filtered must be very small compared with that swept by the long setae of the mandible and the quantitative investigations of Marshall & Orr (1956) into the feeding of nauplii show that very small particles, which would best be filtered in this way, are less efficiently collected than larger ones (15–20 μ) which can be seen to be captured by the sweeping action of the mandibles. In this context the relevant particle size of *Skeletonema* is not its cell diameter, 4–5 μ, but the size of a chain of cells, the length of which is much greater. The nauplii therefore of both calanoid and cyclopoid copepods collect their food in essentially the same way as the nauplius of *Balanus* and Storch is wrong in attributing different feeding method to the two copepod groups.

Storch goes on to quote Claus' description (1858) of the feeding of the nauplius of *Canthocamptus* (Harpacticoida). Here the endopods of the antennae and the endites of the mandibles have become adapted for the prehension and manipulation of large particles obtained from the surfaces over which these nauplii crawl. The nauplii of the Harpacticoida are very uniform in structure (Brian 1921, Gurney 1931) and it is likely that Storch's remarks about *Canthocamptus* apply to most nauplii in the group. Since they are clearly specialised in this respect, the feeding methods of the Harpacticoida are less relevent to the understanding of the feeding methods of other crustacean larvae, than those of the less specialised nauplii of other copepods and cirripedes.

Apart from the nauplii which do not feed (Penaeidea, Euphausiidea, and some Copepoda like *Euchaeta*) and the clearly specialised nauplii of the Harpacticoida, two methods of feeding have been shown to occur in nauplii, that of the branchiopod nauplii and that found in the Cirripedia and cyclopoid and calanoid copepods. In both food particles are collected by a sort of sweep net formed by setae of one pair of limbs, and are then carried to the mouth by two groups of short stout setae, one on the mandibles, the other on the antennae. The notable difference is that in the branchiopod nauplii, the antennae form the food collecting apparatus while in the other nauplii it is the mandibles. The method of transfer of food to the mouth is the same and there is a striking similarity between the form and distribution of the stout setulose setae of the mandibles of *Artemia* for example and the functionally corresponding setae of the mandible of *Calanus*. However the marked difference between the two types of nauplii in the form and distribution of the setae used for food collection seems to suggest that the two methods of feeding have evolved in parallel rather than that one is derived from the other, a suggestion which is in keeping with the generally isolated position of the Branchiopoda among the Crustacea.

SUMMARY

An account is given of swimming and feeding in the nauplii of *Artemia*, *Balanus*, *Calanus* and *Oithona*, with observations on other nauplii. Previous work is reviewed.

In all cases the antennae are the principal locomotion organs. The antennules may play little or no part in locomotion and are mainly balancing organs.

In *Artemia* the antennae are also the chief food collecting organs. Particles are swept into the oral region by the antennal setae and then carried to the mouth by spines on the reduced mandibles.

In *Balanus* and most planktonic copepods, the principal food collecting appendage is the mandible ; particles are swept into the oral region by its long ventral setae and passed to the mouth by spines like those of *Artemia*.

The nauplii of Penaeidea and Euphausiacea and of *Euchaeta* (Calanoida) do not feed.

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THE DISTRIBUTION OF THE BLOOD FROM THE HEART IN SOME AMPHIBIA

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(With 1 plate and 6 figures in the text)

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INTRODUCTION

That account of the circulation and distribution of the blood in the anuran Amphibia commonly given in text-books and generally referred to as the 'classical hypothesis' ascribes the selection and distribution of 'venous' and 'oxygenated' blood to the arterial arches to a mechanism involving (*a*) the spiral valve in the conus arteriosus, (*b*) substantial differences of resistance to flow in the arterial arches, and (*c*) the specific manner in which the blood lies in and flows from the ventricle.

Of the authors who have investigated this problem experimentally a majority agrees that a selective distribution of the blood occurs, but there is little agreement on the mechanism by which it is brought about. The minority of authors who have concluded that the blood is distributed non-selectively are themselves divided: Foxon (1947) considered that the 'oxygenated' and 'venous' portions of the blood were distributed at random to the arterial arches, while Vandervael (1933) thought that both fractions were completely mixed in the ventricle so that the arterial arches each received blood of the same quality.

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In the Australian frog, *Hyla caerulea*, it was found by means of a technique involving the injection of a dye into the blood stream (Simons, 1953 ; Simons & Michaelis, 1953) that, while some experiments showed a selective distribution of the blood from the heart, others showed a ' mixed ' distribution as had been described by Vandervael. It was considered that in this species the distribution, which was conceived as being basically selective, might change to a ' mixed ' one under certain circumstances such as the increase or decrease in the importance of pulmonary breathing. More recently de Graaf (1957) found a selective distribution to occur in *Xenopus laevis* despite the unexpected result that the pulmo-cutaneous circulation received more than half of the ventricular output.

In view of the conflict and uncertainty of opinion, it was considered desirable that experiments similar to those performed on *Hyla* should be repeated using *Rana temporaria*, which is the animal most frequently used by previous authors. This paper presents the results of such experiments, together with the observations of additional experiments on the toad, *Bufo bufo* and the urodeles, *Salamandra maculosa* and *Triturus cristatus*.

EXPERIMENTAL

Materials and Methods

(a) *Specimens*

Animals used were mature specimens taken throughout the year from laboratory stocks. The average room-temperature was 18–19°C and the heart rates of animals during the experiment ranged from 40 to 65 heart beats per minute.

(b) *Operative*

The animal was anaesthetised lightly with ether and placed on its back on a small dissecting board. The body cavity was opened by mid-ventral incision and the pectoral girdle bisected and cut back to display the heart and arterial arches.

The two hypodermic needles of the micro-injection apparatus were introduced, one into the venous return to the heart and one into the pulmonary return, using the procedure described by Foxon (1947). The needle introducing the dye to the pulmonary return was inserted into the pulmonary vein of the left lung and the needle introducing the dye into the venous return was inserted into the great cutaneous vein of the right side.

A third needle (size 20 g.) was then introduced into the forward portion of the anterior abdominal vein. This needle delivered a continuous supply of Ringer's solution from a reservoir at a pressure of 2 cm. Ringer. As soon as this needle had been inserted and fixed into position, the posterior section of the anterior abdominal vein was cut. In a few minutes the blood was thus drained from the animal and its place taken by Ringer's solution. The effect of this was to leave the blood vessels colourless.

Having been thus prepared the animal was used either for direct observation or for photography.

Direct visual observations were made through a binocular microscope using reflected light from a standard ' Pointolite ' microscope lamp or, in some cases, Foxon's (1947) ' transillumination ' apparatus.

If a cine-photographic record was being made, the camera was focused and adjusted so that the conus arteriosus was viewed from the most desirable position. The rhythm of the heartbeat was used for timing : The camera and lights were switched on at the diastole of the atrium and at the next diastole the dye was injected.

(c) *Micro-injection apparatus and dye medium*

The micro-injection units consisted of two " Agla " micrometer syringes used as described by Foxon (1947) with the exception that each was connected to its injecting needle by a short length of fine bore polythene tubing which, though flexible, was non-elastic. By means of the markings on the micrometer scale the dye was injected into the blood stream in specific quantities of 0·001 ml., 0·002 ml., 0·003 ml., etc.

The dye used was Evans Blue which was made up as a 1% solution in Ringer's solution.

(d) *Photographic*

Two cine-cameras were available, one a Cine-Kodak Special used at 64 frames per second, the other a G.45 Aircraft Gun Camera used, after some adjustment of the motor, at 36 frames per second.

The film used was 16 mm. black and white, either Cine-Kodak Super X Reversal or Ilford H.P.3 Negative.

Observations and Results

The nomenclature of the parts of the heart and vessels referred to in the following is explained by reference to Fig. 1.

1. *The auricles*

Dye injected by way of one of the pulmonary veins behaved in similar ways in each type of animal. In the frogs and urodeles amounts of 0·003 ml. (and more) appeared to cloud the entire left auricle as it expanded, but in the toads the amount required to obtain the same effect was greater, 0·004 ml. When smaller amounts of dye were injected (0·002 and 0·001 ml. in the frogs and urodeles, and 0·003, 0·002 and 0·001 ml. in the toads) they entered the auricle and billowed out to form a cloud which, depending on the amount injected, occupied a greater or smaller volume of the expanded auricle. The centres of the clouds of dye were not constant (even in the one animal) in the position they took up in the auricle. The smaller the amount of dye injected the more was it noticeable that it positioned itself in the auricle with an apparent random eccentricity. The cloud of dye remained momentarily suspended in the auricle until it was driven down into the ventricle by the auricular contraction.

When injected by way of the venous return, the dye in all species and in all quantities appeared to fill the expanded right auricle. The intensity of the shadow was, however, dependent upon the amount injected. The difference in appearance of the dye in the two auricles is due, in the case of an injection into the venous return, to its being mingled with the other streams returning to the sinus venosus before entering the right auricle, whereas the dye entering the left auricle from one pulmonary vein meets only the other equal stream from the other lung.

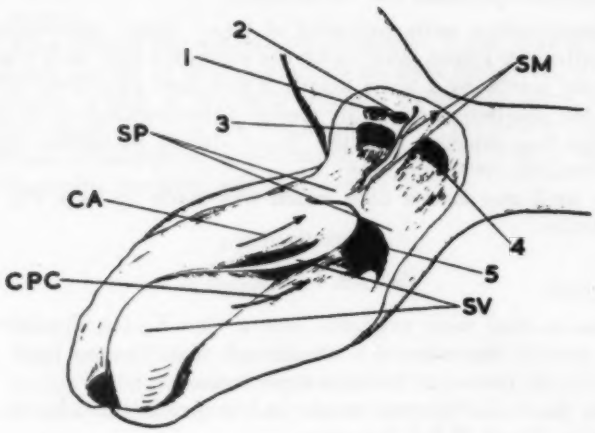

Fig. 1.—General view of the conus arteriosus of the frog dissected from the ventral surface to display the relationship of the internal passages.

C.A. Cavum aorticum ; C.P.C. cavum pulmo-cutaneum ; S.M. septum mediale ; S.P. septum principale ; S.V. spiral valve ; 1 and 2, entrances to the right and left carotid arches ; 3, entrance to the right systemic arch ; 4, entrance to left systemic arch ; 5, continuation of cavum pulmo-cutaneum dorsal to the septum principale leading to the common entrance of the right and left pulmo-cutaneous arches.

2. *The ventricle*

Dye entering the ventricle came to lie in the half corresponding to the auricle through which it had passed. Irrespective of the amount injected, dye lying in the right hand side of the ventricle occupied the greater part of that half, whereas small amounts (0·001 ml.) introduced through the pulmonary return were seen as dark patches occupying only part of the left side. Amounts of 0·002 ml. (and more) darkened the greater part of the left half of the ventricle in all species. Foxon (1951) who followed, by radiography, the course of the radio-opaque medium "Thorotrast" injected into a pulmonary vein, concluded that blood from the left auricle came to lie in the left side of the ventricle and was not mixed thoroughly with blood from the right auricle as Vandervael (1933) had supposed.

3. *The conus arteriosus*

The appearance of streams of dye as they passed through the conus arteriosus of the frog and the toad was seen to differ from that seen in the urodeles.

(*a*) *Rana and Bufo*

When introduced through the pulmonary return and the amount injected small (0·001 ml.), one of three things occurred : either the dye moved along the cavum aorticum (Fig. 2 a) or, having entered the base of the cavum aorticum, it then passed across the free edge of the spiral valve to flow in the cavum pulmo-cutaneum (Fig. 2 b). The third type of appearance was a combination of these first two, that is, the dye entered the base of the conus arteriosus and thereupon split into two streams, one passing along the cavum aorticum while the second passed across the free edge of the spiral valve (see Fig. 2 c).

When the amount of dye injected was increased (0·002 ml. in the frog and 0·002 and 0·003 ml. in the toad) the appearance of the streams in the conus arteriousus was constantly seen as the third type, that is, a split stream emanating from the base of the cavum aorticum. When amounts greater than these were injected the base of the conus became entirely clouded with dye and the distinct streams were obliterated.

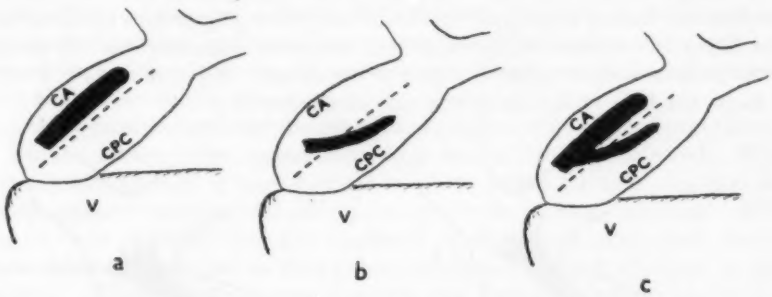

Fig. 2.—Diagram representing the configuration of dye streams in the conus arteriosus of the frog following injection of dye into the pulmonary vein.

a and b, alternative configurations following injection of smallest amounts (0·001 ml) ; c, pattern seen when greater amounts are injected. C.A. cavum aorticum ; C.P.C. cavum pulmo-cutaneum ; V ventricle. The position of the free edge of the spiral valve is represented by the broken line.

Dye introduced through the venous return appeared at the base of the conus arteriosus and flowed in the cavum pulmo-cutaneum only (Fig. 3 a). When amounts of 0·003 ml. and greater were injected, a fainter stream could be seen flowing in the cavum aorticum (Fig. 3 b), but it was not until volumes of 0·005 ml. were injected that a split stream emanating from this cavum was detected (see Fig. 3 c). Beyond these quantities the conus arteriousus, as in the case of large quantities injected by way of the pulmonary return, became clouded over and the distinct streams obliterated.

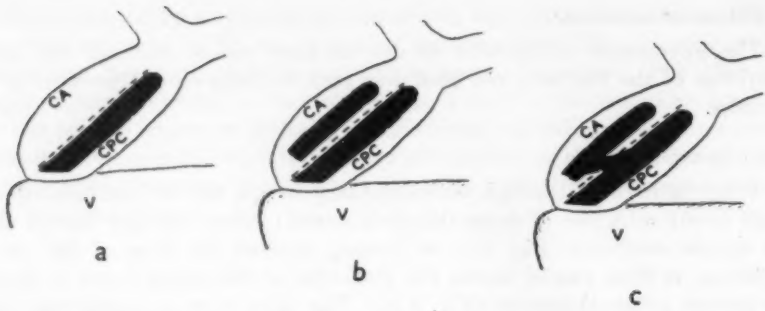

Fig. 3.—The configuration of the dye streams in the conus arteriosus of the frog following injection of dye through the venous return to the heart.
a, pattern following injection of small amounts (0·001 and 0·002 ml); b, pattern following injection of intermediate amounts (0·003 and 0·004 ml); c, pattern following injection of greater volumes. (Identifying lettering as in fig. 2.)

(*b*) *Salamandra and Triturus*

On ventricular contraction dye lying to the right in the ventricle was seen to enter the base of the conus and spiral clockwise (regarded from the ventricle) to form a stream which passed along the ventral half of the conus (Fig. 4 a). On passing through the constriction at the anterior end of the conus into the truncus, the stream tended to ' fan out ' and cover all portions of the truncus. The larger the amount of dye injected, the more complete was the clouding of the truncus and the anterior region of the conus. With amounts of 0·003 ml. or more, the base of the conus was also clouded over.

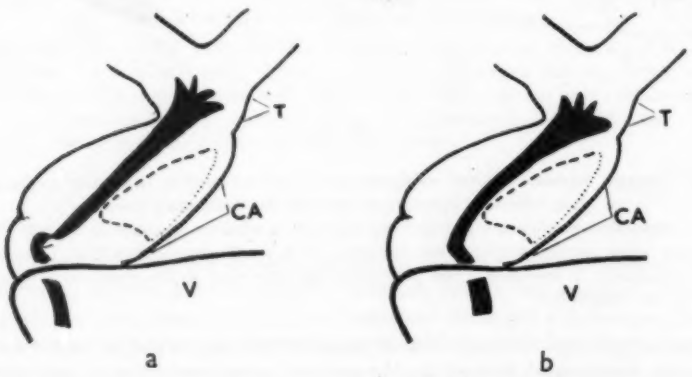

Fig. 4.—The pattern of dye streams in the conus arteriosus of the salamander following injection of dye; a, via the venous return; b, via the pulmonary vein. C.A. conus arteriosus; T truncus, V ventricle.

Dye introduced by way of the left auricle entered the conus from the left side of the ventricle and appeared also to spiral clockwise so that it passed

along the conus in the dorsal region (Fig. 4 b). On reaching the region of the truncus it too spread out to cloud the entire region.

In *Salamandra*, dye whether introduced through either the pulmonary or venous returns, was seen occasionally to pass on both sides of the small spiral valve.

4. *The arterial arches*

The flow of dye in the arterial arches of the urodeles was not examined because of the difficulty of displaying the vessels satisfactorily.

In the frog and toad the distribution of dye to the arterial arches was found to depend upon the amount injected. Thus, in a series of observations on the distribution of dye to the arterial arches of twelve frogs, examined with the aid of the 'trans-illumination' apparatus, dye which had been introduced through the pulmonary return was seen to be distributed as follows: when 0·005 ml. was injected, it was found always to flow in all three pairs of arches. On thirteen occasions 0·004 ml. was injected and the dye appeared in all three arches eleven times and in both carotid and both systemic arches twice. When the amount injected was reduced to 0·003 ml. all three pairs of arches received dye on four occasions while both carotid and both systemic arches received it on nine. When 0·002 ml. was injected the dye appeared in both carotid and both systemic arches on eleven occasions, in both carotid and right systemic vessels twice and in both carotid arches alone once. When 0·001 ml. was injected, both carotid and right systemic arches received dye on eight occasions, both carotid and both systemic arches once, the carotids alone on six occasions and the left systemic vessel alone also on six occasions.

In these same animals the distribution of the dye, when introduced through the venous return, also varied according to the amount injected. When 0·005 ml. was injected, it was observed in all the arches on eight occasions and in both pulmo-cutaneous and left systemic vessels on two occasions. When 0·004 ml. was injected, the dye appeared in all arches once, both pulmo-cutaneous alone once and in both pulmo-cutaneous and left systemic on nine occasions. When 0·003 ml. was injected, the dye appeared in the pulmo-cutaneous vessels alone on nine occasions and in the pulmo-cutaneous and left systemic on four occasions. When 0·002 ml. was introduced, the resulting faint shadow produced in the arterial arches was confined to the pulmo-cutaneous arches (ten occasions) with the exception of one occasion when a shadow also appeared in the left systemic arch.

In the toad similar results were seen. When the volume of dye introduced by the pulmonary return was 0·004 ml. or less, it was seen only in the carotid and systemic arches and behaved in much the same way as the smaller volumes in the frog. When introduced through the venous return, volumes of less than 0·006 ml. were restricted to the pulmo-cutaneous arches.

5. *Time of movement of dye streams*

In all the observations which were made in the course of this work the impression gained was that dye, whether introduced through the venous or

pulmonary returns, entered the base of the conus arteriosus at the same moment of the cardiac cycle. That is to say, contents from the right and left sides of the ventricle entered the conus simultaneously. Again, when examining the flow of dye streams in the arterial arches, it was always the case that, when dye flowed into more than one pair of arches, it did so simultaneously. These impressions of simultaneous movement were confirmed when the cine films made of the flow of dye through the hearts of eleven frogs were analysed (see Plate).

DISCUSSION

It is now generally agreed that in the hearts of Anura the blood which enters the ventricle from the left and right auricles tends to keep to the same sides of the ventricle with some mixture occurring in the central part. The only author who has stated that he believed complete mixing occurred in the ventricle is Vandervael (1933). The point at issue is how much mixing of the blood takes place on ventricular contraction. Foxon (1947, 1951) thought that mixing at this stage might be considerable. The ' classical hypothesis ', however, assumes that the blood from the right side of the ventricle is expelled first and sent to the pulmo-cutaneous arches while blood from the left side is expelled last and is sent to the carotid arches. That is, that blood from the two auricles is kept separate by passing through the conus at different times.

As has been mentioned in the introduction to this paper, varying results had been obtained in *Hyla caerulea* : sometimes the distribution appeared to be selective and sometimes ' mixed '. When the results of the present work on *Rana temporaria* (and *Bufo bufo*) are surveyed it is clear that, although at first sight the observations made when 0·001 ml. of dye was injected by way of the pulmonary vessels into the left auricle (the distribution being : carotid and right systemic arches 8, carotids and both systemic arches 1, carotids alone 6, left systemic alone 6) might suggest a form of random distribution, this is not so, for the pulmo-cutaneous arches never received the dye. Also the carotid arches received the dye with the greatest frequency, the combination of carotid and right systemic arches was next in order of frequency and the left systemic arch alone last. Thus this is evidence for selective distribution. When the results of the injection of larger volumes of dye are considered, the evidence for selective distribution is reinforced since it becomes clear that blood from the left side of the ventricle tends to pass to the carotid and right systemic arches, while blood from the central part of the ventricle tends to pass to the left systemic arch, and blood from the right-hand side to the pulmo-cutaneous arches. There is, however, some variation in the behaviour of the dye, as for instance on those occasions when volumes of 0·001 ml. injected into the pulmonary vein appeared in the left systemic arch. This can be explained by supposing that on these occasions the dye, on entering the ventricle, became located in the more central region of it instead of the left-hand region, which is the position it usually assumed.

This interpretation becomes more conclusive when the results of injections through the venous return to the right auricle are considered. They lead to the conclusion that there is a physiological connection between the left side

of the heart and the carotid and right systemic arches on the one hand, and the right side of the heart and the pulmo-cutaneous arches on the other. The left systemic arch appears to be intermediate in character and is served both from right and left sides of the ventricle. It is obvious that these relationships and the resulting selective distribution of the blood differ from those envisaged in the ' classical hypothesis '.

Conclusions equally distinct from the ' classical hypothesis ' have recently been drawn by de Graaf (1957) in respect of *Xenopus laevis*. He found that in this toad a selective distribution occurs but its pattern is unique and is such that, while blood from the right auricle supplies only the pulmo-cutaneous vessels, the blood from the left auricle passes into all three pairs of arterial arches. This pattern of distribution he found to be associated with a large pulmo-cutaneous circulation which at each heartbeat received more than half of the total cardiac output.

In several species of the Genera *Rana*, *Bufo* and *Hyla*, some authors (Acolat, 1938 ; Savolin, 1948 ; Simon & Michaelis, 1953) have commented on the relative proportions of ' venous ' and ' oxygenated ' blood carried by the pulmo-cutaneous vessels, but none has found that the volume carried exceeds the amount carried by the remaining arches. It is therefore of interest to find that in *Xenopus* this unusual circulation does not preclude a selective distribution. One must conclude that in the anuran heart the precise pattern of the distribution, although basically selective, varies with the species.

A point which was made in earlier accounts of the distribution of the blood (see, for instance, Sedgwick, 1905) was that there was a qualitative difference between blood flowing in the right systemic arch on the one hand and that flowing in the left on the other hand. Frechkop (1955) substantiated Ecker's (1889) finding that in *Rana* there is an anatomical difference between the two systemic arches, and it is of some interest to note that this difference is reflected in the present work, where it was found that the dye streams entered the left systemic arch from both cavae of the conus arteriosus but entered the right systemic arch only from the cavum aorticum.

The firm indication of a selective distribution given in the present work is in marked contrast to the conflict and confusion of previous work. The reason for this is that in the present work the dye was non-particulate, whereas previous workers have used either a particulate substance (Indian ink, Algae, starch particles) or a viscous medium (" Thorotrast ") which apparently has not flowed smoothly through the chambers of the heart. To this must be added the further circumstance that, by means of a fine bore non-elastic tubing, the amount of dye injected could be controlled with precision.

Having established that there is a selective distribution in *Rana* and *Bufo*, it is now necessary to account for it.

No recent author has given any account of the alleged time difference of the passage of the two moieties of blood through the conus. Noble (1925) and Ozorio de Almeida (1923) both stated that the movement of blood in the two passages of the conus was simultaneous. Vandervael (1933), Foxon (1947, 1951) and de Graaf (1957) found that injected material passed on both

sides of the spiral valve simultaneously. Similar results were found in the present experiments and, furthermore, the registration of the pressure pulses in the arterial arches of *Rana* (Simons, 1957) and of *Xenopus* (de Graaf, 1957) indicates that movement of the blood in them is simultaneous. It must therefore be contended that that part of the 'classical hypothesis' which accounts for selective distribution in terms of differences in time of flow of the blood into the conus and arterial arches is incorrect.

A point which must be stressed is that in the present work not only do the 'venous' and 'oxygenated' streams arrive simultaneously at the base of the conus, but also in their correct relationship to the entrances to the cavum aorticum and cavum pulmo-cutaneum. In *Xenopus* de Graaf (personal communication) found a similar state of affairs. The problem is to explain how the blood entering the conus from the left side of the ventricle comes to lie in a predominantly dorsal position, while the right-side blood finds itself ventrally placed. I discussed this problem with the late Mr de Graaf and we were led to conclude that the contents of the ventricle, as they are expelled into the conus arteriosus, are given a right-handed spiral movement.

Such a spiral movement was observed in the urodeles examined, but in the frog and toad the position of the base of the conus arteriosus, made it impossible to identify a similar movement of the blood in the anuran heart. In *Triturus*, the spiral movement obviously does not depend upon the presence of a spiral valve in the conus, and in *Salamandra* the spiral valve, being small and at a distance from the pylangium, cannot be regarded as the cause of the movement. It would thus seem that the deflection of the blood from the right side of the ventricle towards the ventral sector of the conus, and the corresponding deflection of the blood from the left side of the ventricle to the dorsal sector of the conus, is achieved in some other way.

In this connection it is interesting to note that spiral streaming of precisely this pattern is present in the preconal region of embryonic hearts. Bremer (1932) reported it in the heart of the 48-hour chick and Romhanyi (1952) found that in a model of the human bulbus cordis spiral movement occurred. Goerttler (1955), by means of experiments on a series of glass models of the developing human heart at different stages, showed that the spiral streams continued throughout development. In the fully developed amphibian heart the helical motion of the blood passing from the ventricle must be a property retained from the embryonic stages and the underlying mechanism of the motion is most probably the helical arrangement of the muscle bundles (see Benninghoff, 1933). Again, in the anuran heart the entrance to the conus arteriosus from the ventricle is, as Acolat (1938 b) pointed out, more horseshoe-like than circular and this would facilitate the spiral movement of the blood.

The acceptance of the possibility that the blood leaving the ventricle is already describing a right-hand helix as it comes into the region of the spiral valve allows the resolution of the conflict betwen results of previous work and an explanation of the results reported in the present work. It also allows the formulation of a rational account of the selective distribution in *Rana* and *Bufo*. This may be summarised in the following way (see also Fig. 5).

Blood from the right and left auricles enters the ventricle and comes to lie in the right and left halves respectively. A certain degree of mixing of the two moieties takes place in the middle region of the ventricle. On leaving the ventricle the blood executes a right-hand spiral so that the right ventricular blood becomes directed towards the crescent-shaped opening of the cavum pulmo-cutaneum, while the blood from the left side of the ventricle becomes directed into the entrance to the cavum aorticum. The opening to the cavum aorticum is, however, larger than that to the pulmonary ramp and therefore also receives a good deal of the blood from the middle region of the ventricle.

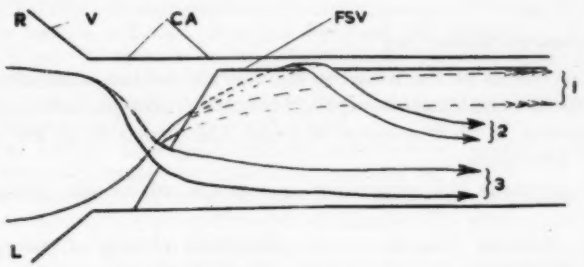

Fig. 5.—Diagrammatic representation of the flow of blood from the anuran ventricle into the conus arteriosus. The cavum aorticum is imagined as being below the plane of the paper, the spiral valve as being in it and the cavum pulmo-cutaneum as being above it. Because of a helical movement as it leaves the ventricle, blood lying in the right region flows mainly into the cavum pulmo-cutaneum (flow-lines 3). Blood lying in the left region flows mainly into the cavum aorticum (flow-lines 1). Some blood from the middle region goes directly into the cavum pulmo-cutaneum while the rest flows into the cavum aorticum where some of it may pass across the free edge of the spiral valve (flow-lines 2) to form the more ventral laminae flowing in the cavum pulmo-cutaneum.

C.A. conus arteriosus; F.S.V. free edge of spiral valve; L and R left and right sides of V–ventricle.

Once the ' venous ' stream has entered the cavum pulmo-cutaneum it has no option but to proceed along it, but the stream which has entered the cavum aorticum has two possibilities. The main mass proceeds along the length of the cavum aorticum and enters the carotid and right systemic arches, while part of it splits off to pass across the free edge of the spiral valve and flow in the cavum pulmo-cutaneum ventral to the stream which was originally contributing to the same cavum. This stream, together with some of the 'venous' blood already flowing in the cavum pulmo-cutaneum, enters the left systemic arch, the entrance to which lies in the left semi-chamber of the truncus formed by the *septum mediale*. The remainder of the blood in the cavum pulmo-cutaneum flows into the pulmo-cutaneous vessels. Thus, while the arterial arches receive their fractions of blood simultaneously, they receive it selectively, the carotid and right systemic arches receiving the greater part of the left auricular blood, the left systemic arch receiving mixed blood from the middle and the right regions of the ventricle, and the pulmocutaneous vessels receiving the greater part of the right auricular blood.

SUMMARY AND CONCLUSIONS

Experiments are described in which the course of a dye (Evans Blue) introduced through the venous and pulmonary returns of the hearts of various amphibians was observed. The principal animal used was the frog, *Rana temporaria*, with additional observations made using the toad, *Bufo bufo*, and the urodeles *Triturus cristatus* and *Salamandra maculosa*. It was found that the behaviour of the dye passing through the conus arteriosus and into the arterial arches depended on the volume injected and that this may account for the conflict of opinion emerging from previous work. A new account of the mechanism of the selective distribution of the blood in the Anura is advanced.

The conclusions drawn are :—

1. The movement of the blood in the arterial arches is simultaneous.

2. In all species the contents expelled from the right and left auricles remain tolerably separate in their passage through the ventricle as far as the base of the conus arteriosus.

3. In the anurans the separation is maintained by the presence of the spiral valve.

4. In the urodeles, because of the reduction or lack of the spiral valve, the streams of ' venous ' and ' oxygenated ' blood become mixed by the time they reach the truncus and therefore the arterial arches receive blood of the same degree of oxygenation.

5. In the anurans most of the right auricular blood enters the cavum pulmo-cutaneum, while all the left auricular blood, together with some of the ' mixed ' blood from the middle region of the ventricle, enters the cavum aorticum to be sent, in the main, to the carotid and right systemic arches.

6. These entrances occur simultaneously and can be explained by assuming that the blood as it leaves the ventricle undergoes a right-hand helical deflection.

7. After entering the cavum aorticum some of the blood (mainly ' mixed ' blood) passes across the free edge of the spiral valve and enters the cavum pulmo-cutaneum, whence it flows into the left systemic arch, together with ' venous ' blood which was contributed directly into the cavum (see 5 above).

8. The proportions of ' oxygenated ', ' mixed ' and ' venous ' blood in the cavae of the conus probably vary slightly from moment to moment, according to the prevailing conditions of resistance to flow in the pulmo-cutaneous vessels, but this does not seriously upset the basic pattern of the selective distribution.

ACKNOWLEDGMENTS

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EXPLANATION OF THE PLATE

Strips from cine-records showing the difference in the pattern of dye flow in the conus arteriosus of the hearts of two frogs following the injection of dye through the alternative routes of the pulmonary and venous returns. Film speed : 36 frames per sec.

Strip A.—Frog 1 (Injection of 0·002 ml. dye through the pulmonary vein). The dye is seen in the left auricle (frame 1) which forms most of the dark bulging shadow in the lower right region of the frame. The conus arteriosus lies along the border of this shadow and points towards the upper right of the frame (in reality it recedes from the camera). Along part of the (actual) left-hand side of the conus there is seen a region of pigmentation (seen more clearly in focus in strip B). Immediately above this area the arterial arches are seen—the left arterial arches pass off the right edge of the frame and are not individually distinguishable, while the right arterial arches pass across towards the upper left corner of the frame.

The dye appears in the conus in frame 2 as a dark patch ' opposite ' the pigmented region. It also shows faintly in the right systemic arch. In frame 3 the dye shadow in

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the conus is extended along the right border (of the conus) towards the proximal region. The dye is also apparent in the carotid arch. In frame 4, the dye is intensified in the carotid and systemic arches but is almost gone from the conus.

Strip B.—Frog 1 (Injection of 0·002 ml. dye through the venous return). In frame 1 the dye appears in the conus arteriosus as a dark band in the proximal region close to the ventricle. In the successive frames the dye is intensified. It will be seen that the stream in this instance flows 'below' the pigmented area and not 'opposite' as in Strip A. At no time is there any sign of dye in the arterial arches.

Strip C.—Frog 2 (Injection of 0·003 ml. dye through the pulmonary vein). The conus arteriosus of this heart lies in the elliptical whitish region in the middle of the frame. Its proximal end is marked by a dark patch almost mid-way between the frames and approximately ¼ the width of the frame in from the left side of the strip. This dark spot is the shadow of the orifice leading into the conus from the ventricle as seen through the almost transparent wall of the conus. Distally the conus points towards the right corner of the frame and gives rise to the arterial arches which, because of the camera angle, cannot be distinguished individually on either side.

In frame 2 dye is seen entering the conus as a dark crescent massed to the right and mainly above the ventriculoconal orifice.

In frame 3 the dye has passed along the cavum aorticum (thus out-lining the upper, as seen in the photograph, edge of the conus). A thin stream of dye has also passed across the free-edge of the spiral valve (seen as a whitish blur under the dye), it passes in the direction of the lower right of the frame and then curves towards the upper right.

In frame 4 the stream across the spiral valve has become fainter but a continuation of it can be seen in the left systemic arch. The dye in the cavum aorticum has become intensified.

Strip D.—Frog 2 (Injection of 0·003 ml. dye through the venous return). In frame 2 the dye enters the conus massed mainly below the position seen in the corresponding frame in strip C. It travels forwards and in frame 3 can be seen to have entered mainly the cavum pulmo-cutaneum (thus out-lining the lower border of the conus). Some dye however has also entered the cavum aorticum and the configuration forms a horse-shoe shaped shadow quite different from the pattern seen in the companion strip.

In frame 4 the dye has extended right along the conus and shows as a comparatively wide shadow in the cavum pulmo-cutaneum and as a narrower, lighter stream in the cavum aorticum.

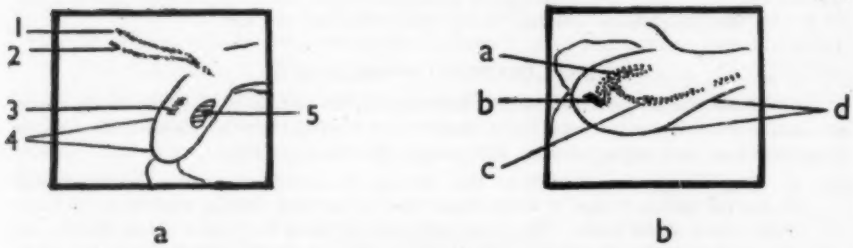

Fig. 6.—Outline drawings for orientating the field of: a—Frame 4, Strip A. b—Frame 4, Strip C.

1. dye in right carotid arch.
2. dye in right systemic arch.
3. dye in cavum aorticum.
4. proximal end of conus arteriosus.
5. pigmented area referred to in explanation.

a. dye in cavum aorticum.
b. shadow of ventriculo-conal aperture.
c. dye passing across free edge of spiral valve.
d. border of conus arteriosus.

The distribution of the blood from the heart in some amphibia.

PROTEAN DISPLAYS: A FORM OF ALLAESTHETIC BEHAVIOUR

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The theory of natural selection, whose centenary we are celebrating this year, gave rise to a number of new special subjects. One of these, which has continued to arouse great interest, is the function and evolution of allaesthetic displays, that is, properties of animals, (structures, colours, movements, postures, vocalisations, etc.), which produce behavioural effects on other animals, of the same or other species, and can be selected on this basis. Colours, in particular, were early classified from this point of view (Wallace, 1889; Poulton, 1890), and the classification was gradually extended to other properties. Rich illustration of various types of display was provided in the great book of Cott (1940).

A rational classification of allaesthetic properties must depend on the analysis of behaviour in the reacting animal (Cott, 1954; De Ruiter, 1955). Such analysis has barely begun experimentally in the predator-prey context; for instance, Thayer's theory of counter-shading was published in the nineteenth century, but De Ruiter's was virtually the first study of predator behaviour in reaction to it (1955). The older classifications were based on a somewhat anthropomorphic view of behavioural mechanisms in the reactor (quite properly, for want of better information); it is already apparent that these early categories are beginning to lose their usefulness (cf. Cott, 1954). Classification must continue to be provisional in the context of predator-prey relations; but at least we can begin to relate it to the properties of animal behaviour revealed by ethologists, which have already thrown so much light on social allaesthetic displays (Tinbergen, 1948; Morris, 1956).

Any such categories must take into account an important dichotomy within behavioural mechanisms. Internal factors apart, the mood of an animal is determined by one set of environmental factors (releasing stimuli), while the orientation of its released reactions is determined by a second set (directing stimuli). These sets do not necessarily coincide, and reflect a fundamental dichotomy into behavioural mechanisms of determination and adjustment (Tinbergen, 1942, 1948, 1951; Baerends, 1941; Russell *et al.*, 1954). A prey animal may avoid capture either by influencing the mood of the predator, or by impairing the latter's ability to orient its reactions. In the former case, it may act positively by releasing a drive other than hunting (e.g. escape), or negatively

by denying stimuli for the release of a capture reaction, as must often be the significance of cryptic coloration (cf. De Ruiter, 1955). In the latter case, the prey animal may, for instance, divert attack away from its own body, or at least from the most vulnerable parts of this. Similar considerations apply to the distraction displays by which an animal may thwart predators of its young.

The mechanisms which release reactions or changes of mood are themselves of two different kinds. In some cases, an animal has become familiarised with an object, so that its releasing mechanism is related to the latter closely and in detail. The reaction can now be released only by the proper object, or by a model of very high fidelity, closely resembling the object in all respects. In other cases, the reaction is geared to a few key stimulus properties of the biologically relevant object, which can now be replaced by a model of very poor fidelity, but which carries the key properties. In such cases it is possible to construct supernormal models, quite unlike the original object but even more effective in their action on the releasing mechanism (Tinbergen, 1942, 1948, 1951; Russell, 1957).

Owing to the limited capacity of the genetic channel for information transmission, familiarity mechanisms are usually developed in ontogeny. Key stimulus mechanisms may either be innate or acquired by conditioning processes, since Diebschlag was able to construct supernormal models for conditioned releasing mechanisms (Diebschlag, 1941; Russell & Russell, 1957; Russell, in press).

In the context of displays by prey, the distinction becomes important. Chance (1957) has introduced the concept of biological efficiency—a measure of relative frequency with which, in the course of an animal's life, a reaction occurs with biologically appropriate results. As he points out, the degree and kind of efficiency likely to be selected for may differ in different reactions (cf. also Hayes *et al.*, 1953). We may enquire under what conditions an animal will be content with key stimuli, and when familiarity will be necessary. The following hypothesis may be suggested. Key stimulus mechanisms are used when it is specially important not to lose a chance of reacting to the right object, while reactions to the wrong object are of minor disadvantage. (Sometimes the indiscriminateness is corrected by a second reaction stage with different key stimuli—Russell, 1952, 1954). Familiarity is employed when it is specially important not to react to the wrong object. This hypothesis accords well with the experimental results of Diebschlag (1941).

Structures (etc.) specially selected on account of their stimulus properties for other animals are known as releasers; if the other animals concerned are of the same species, we speak of social releasers (Lorenz, 1935; Tinbergen, 1948; Baerends, 1950). Not all stimuli are releasers: thus the short neck of the hawks, a key stimulus for escape in their prey species, was obviously not evolved in relation to this function (Tinbergen, 1948). In relations between predators and prey, it is common to find releasers parasitic on the releasing mechanisms of the enemy (Baerends, 1950).

The evolution of displays in prey animals is profoundly influenced by the nature of the predator releasing mechanisms they are evolved to trigger. (And the whole of the following argument is equally relevant to predator

displays related to releasing mechanisms in the prey). Batesian mimicry, for instance, is geared to familiarisation mechanisms which are continually being adjusted in the predator's ontogeny. The result (especially when one species mimics two or more others polymorphically—Ford, 1945) is a complex interaction, in which the course of the mimics' evolution is affected by the relative abundance of the models (Fisher, 1930). On the other hand, eye-spot patterns in the Lepidoptera are probably evolved in relation to key stimulus mechanisms for escape reactions in their predators, which are themselves related initially to widespread properties of the latters' predators. In the Batesian mimicry case, failure to eat tasty insects is approximately as important selectively as waste of time on distasteful ones, and a shifting balance obtains. In the eye-spot case, certainty of escape from dangerous enemies is the over-riding consideration for the predator. Here the Fisher rule does not carry much weight in practice, for an effective minimum abundance of the models can be assumed (cf. Blest, 1957).

The allaesthetic effects usually considered are systematic. They act to induce a particular mood (by denying or providing stimuli for specific releasing mechanisms), or to divert the reactor's performance in a particular wrong direction. The prey thus provide the predator with systematically false information (McCulloch, 1951; Hayes *et al.*, 1953). But just as any systematic code can be cracked, so any systematic display of this kind is exposed to counter-measures by the predator. These can be appreciable in ontogeny, where De Ruiter has shown how predators can correct their initial indifference to the sticks mimicked by some of their prey (1952); doubtless phylogenetic correction is also a factor. The sort of display we have illustrated by the eye-spot pattern is one way round this difficulty, for here counter-measures do not pay the predator. But this device cannot always work, and there is another of great importance which has not been the subject of the attention it deserves.

This is the case of displays which are sufficiently unsystematic to be quite unpredictable in detail and which thus frustrate both ontogenetic and phylogenetic correction on the predator's part (cf. Chance, in press.). Such displays must be of unique advantage in the context of this particular function, though doubtless subject to limitations in others. Instead of deceiving, they confuse. They might quite properly be called cryptic, in the more sophisticated sense of the term, and have been so called provisionally elsewhere (Chance, in press). But the word ' cryptic ', in this context, has been used largely in relation to literal concealment, and almost entirely in relation to systematic deception. We therefore propose the term " Protean Displays ". The mythical Proteus frustrated would-be captors by constantly changing his shape, so that they had nothing systematic to which to react.

A display may be Protean in relation to the predator's orientation mechanisms. Marler (1955) has shown that alarm calls in birds often have the property, not of systematically misleading a predator, but simply of making localisation of the sound difficult or impossible. But a display may be Protean in a more general way, by an effect on the predator's mood. It may then often include the systematic release of escape reactions in the predator, and many Protean displays may be frightening (cf. Holmes, 1940). Sudden, unsystematic

events are frightening for many animals, on much the same grounds as eye-spot patterns. But this may not necessarily be true for predators with few or no dangerous enemies, and in general may not be the only effect.

Protean displays involve, rapid, sudden transitions, as one obvious component of their confusing effect. They are therefore a special case of what Morris (1956) has called "transformation displays" (which also include epigamic ones). The transitions within the individual prey animal are as random and incoherent as possible, none lasting long enough to produce a sustained mood-inducing or orienting effect. The prey animal thus requires an extensive repertoire of sudden, rapid, incoherent movements, which can succeed each other in an indeterminate sequence. These conditions are met in two well-studied cases: the colour changes of the cuttlefish (Holmes 1940), and the convulsive, seizure-like reactions shown by *Peromyscus* (and probably other small mammals) when they are frightened *and* prevented from systematic escape reactions (Chance, 1954, 1957; in press). The latter include violent, disoriented running, catalepsy and wild aggressive attacks, the components occurring in rapid succession. In both cuttlefish and *Peromyscus*, the displays achieve their Protean nature both by the incoherence of each component and by the rapid, unpredictable transitions from one to another.

The *Peromyscus* case provides one further Protean element, to which Chance has called attention (1957; in press). The seizures do not occur in all individuals of the prey species, which is thus polyethic (i.e. behaviourally diverse, by analogy with the diversity of structure usually referred to in the context of balanced polymorphism—Ford, 1945). The displays thus defeat prediction in three ways: incoherence of each component, incoherence of the sequence, and the fact that the whole display may or may not occur in a particular individual.

Many other examples are probably available. Russell (unpublished observation) repeatedly observed a convulsive, clonic reaction in one particular male individual of the species *Xenopus laevis* (Daudin). This reaction appeared when the frog had been isolated in a jar, where systematic escape by swimming was prevented; it never occurred in the large living tanks where the animals were usually kept. It also never occurred in a hundred or so other individuals observed in both these conditions. The reaction made the animal extremely difficult to handle.

In man, intelligent prediction may be frustrated by confusion or systematic deception. Lower animal predators are, of course, mainly influenced by instinctive interaction (Russell & Russell, 1957; in press), affecting either innate or conditioned releasing mechanisms. The instinctive counterpart of systematic deception we have already considered. The confusion, or high noise level (in the engineers' sense), provided by a Protean display is another matter. What happens to the instinctive mechanisms of a predator faced by such a display is a new and interesting question, which may have important implications for ethology. The Protean displays mentioned are all (subjectively) confusing to human observers, and make the displayers difficult to handle, but we know nothing of the mechanism or nature of their postulated effect on predators (or, in the cuttlefish case, prey—Holmes, 1940). The problem could

perhaps be approached by the use of simple Protean models, such as toy kaleidoscopes.

The evolution of polyethic Protean displays also raises new issues. It is not perfectly analogous with the case of simple cryptic coloration, where some quantitative theory is already available (De Ruiter, 1955). Nor is it perfectly analogous with either Batesian or Mullerian mimicry, so the rules proposed for these cases cannot simply be transferred to this situation. It would be of great interest to know whether the relative frequencies of convulsing individuals vary in different populations of *Peromyscus*, which in nature is often split into small colonies; differences in intensity of predation might arise in such a situation (cf. Sheppard, 1953, 1954; Chance, 1957). For purposes of confusion, it is not easy to see what the optimal ratio would be between convulsing and non-convulsing individuals, since success for the prey species depends both on the effectiveness of the display itself and on its irregular incidence in the prey population. Some empirical results may therefore be necessary to guide theory in this field. We commend to the attention of zoologists both the ethological and the evolutionary problems raised by the occurrence of Protean displays.

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STUDIES ON THE FOOD OF COLLEMBOLA IN A DOUGLAS FIR PLANTATION

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INTRODUCTION

The feeding habits of soil Collembola are the best indication of their role in the breakdown of the plant litter to humus. An attempt has been made to study their food in some detail and to discover as much as possible about food preferences in a single habitat.

The habitat selected was a pure stand of Douglas fir at Waen Wen, near Bangor, North Wales. This plantation had a complete canopy and no ground flora ; a detailed description of this area is given by O'Connor (1957).

Collembola were extracted from the soil profile using a Tullgren funnel. The collections of litter were taken from a number of random localities within the wood to avoid possible local peculiarities in diet.

PREVIOUS WORK ON THE FOOD OF COLLEMBOLA

The first important work on the feeding habits of Collembola was that of Macnamara (1924) who divided them into two groups, the suctorial forms which have no molar plate on the mandible, and the chewing forms which have a well developed mandibular molar plate. He contended that the

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suctorial forms were carnivorous and the chewing forms vegetarian. Macnamara examined the gut contents of Collembola and found them to consist of decaying vegetable matter, fungal hyphae and spores and minute Algae, and suggested that fungi were a favourite food. Other diets which he noted were *Achorutes viaticus* (*Hypogastrura viatica*) feeding on maple syrup, *Isotoma palustris* (*Isotomurus palustris*) and *Sminthurus aquaticus* (*Sminthurides aquaticus*) feeding on diatoms, desmids, and conifer pollen, *Sminthurus viridis* on pollen and living plant tissue, *Achorutes armatus* (*Hypogastrura armata*) feeding on mushrooms and *A. viaticus* on a colloidal sewage deposit.

Womersley (1932) quotes *Neelus sp.* as feeding on Algae and Savely (1939) suggests that Collembola feed on Fungi and decayed wood. Eaton & Chandler (1942) refer to Collembola as " saprophagous " and Eaton (1943) refers to them as feeding upon faeces.

Agrell (1940) examined the gut contents of Collembola in one locality and found all the individuals had the gut packed with fungal hyphae, whilst in another locality the same species had the gut full of amorphous detritus. He concluded from this that the soil Collembola are unspecialised feeders. Various workers have attempted to discover the food preferences of Collembola by offering them different food substances e.g. Strebel (1932), but unfortunately Collembola will thrive on food which is obviously not their natural diet and therefore this type of preference experiment throws little light on their food in nature.

The most valuable work on the food of Collembola is that of Schaller (1949). He found that Collembola would not generally feed on fresh leaves, but that *Tomocerus flavescens* and *Orchesella flavescens* would feed on decaying leaf litter and that the former flourished on this diet. Schaller also fed Collembola on the excrements of larger arthropods e.g. *Glomeris*, *Julus* and *Porcellium* and found that *Tomocerus flavescens* and *Onychiurus armatus* fed on the excrement whereas *Isotoma olivacea* Tullberg would not. He observed Collembola feeding on leaf litter, excrements and fungal hyphae. Schaller also examined collembolan gut contents and found that fungal hyphae were the predominant constituent, although he found decaying wood in some species. From this work he concluded that Collembola play an important role in both the breakdown of litter and of humus and that the micro-fauna is important in the role it plays in the comminution of the humus.

In a recent paper Murphy & Doncaster (1957) have suggested that *Onychiurus armatus* feeds on nematodes of the Genus *Heterodera*. Unfortunately their experiments cannot be regarded as being really conclusive as starved Collembola will feed on almost anything made available to them, including filter paper. Table 1 in their paper shows that *Onychiurus armatus* will feed on *Heterodera* cysts in preference to rootlets, but in the cultures where peat-moss is also present *Heterodera* remained unattacked. It is quite possible that *Heterodera* and other nematodes are an important food of Collembola (see also Brown, 1954) but this can only be determined by carefully controlled preference experiments ; this might well account for the large numbers of Collembola with apparently ' empty ' guts (in lactic acid).

This brief survey of the previous work on the food of soil Collembola suggests that the most important substances are Fungi, decaying plant remains and detritus. The object of this study was to gain some idea of the proportions of the various substances ingested and to discover if different species in the same habitat had different feeding habits.

METHOD

Collembola will feed upon almost any substance which is palatable to them, fungal hyphae, moss protonema, arthropod faeces, decaying leaves, other Collembola (living or dead), filter paper, salt agar, cheese, butter, starch, potato and syrup. Offering different kinds of food to them therefore gives little indication of their natural feeding habits and the very diversity of the food accepted suggests that they are unspecialised feeders.

It was decided that the most reliable approach would be to examine the gut contents of specimens taken directly from the wood. This method may be criticised on the grounds that indigestible substances may appear to be the predominant food whilst rapidly digested materials seem unimportant. This criticism can be partly answered on the grounds that an examination of the different regions of the mid-gut showed no consistent increase in any substance towards the anterior end, and that frequently the gut was so closely packed with fungal hyphae that there can have been space for little else.

The Collembola were killed in 95 per cent alcohol and then transferred to warm 50 per cent lactic acid for at least twenty-four hours ; this clears the Collembola making the solid gut contents clearly visible. The specimens with visible gut contents can then be selected. The gut contents were dissected out on a slide and squashed beneath a cover slip ; in the case of the smaller specimens a squash was made of the whole animal. The gut contents were then examined microscopically.

A botanical stain-mountant known as Steinmetz stain was used initially ; this stains plant tissue in the following way : Cuticle—red, lignin—yellow, cellulose—pale yellow or colourless, mucilage—a white precipitate, starch—blue, aleurone—yellow, oils, resins and latex—red, tannin—milky blue, alkaloids—crystalline, calcium—tufts of needle-like crystals. This method was later relinquished as much of the decayed cellulose was naturally dark yellow or brown in colour and the mountant rapidly deteriorated. Most of the gut contents were therefore mounted in lactic acid or lactophenol. Cotton blue in lactophenol, which is the usual stain for Fungi, was unsatisfactory as it also stained the collembolan tissues.

RESULTS

(a) *Collembola without visible gut contents*

Some species very rarely had any visible gut contents and an example of this is *Friesea mirabilis f. reducta* Cassagnau, of which only one or two individuals in a thousand showed visible food in the gut ; other examples from Waen Wen were *Anurida granaria* Folsom and *Neanura muscorum* (Caroli). These three species differ from the others in the wood in that they

have no molar plate on the mandible. The lack of visible gut contents supports Macnamara's (1924) contention that they are fluid feeders, Macnamara also suggests that these forms are carnivorous ; this second suggestion is difficult to accept as there is little evidence in its favour. It is difficult to tell whether Collembola are naturally carnivorous as any starved specimens will feed upon the corpses of other Collembola. Observations in the laboratory have shown that a specimen of *Isotoma viridis* Bourlet may kill and devour an *Isotoma notabilis* Schaffer yet judging by its gut contents *I. viridis* is normally vegetarian. Some starved specimens of *Friesea mirabilis* were presented with freshly killed specimens of other Collembola but they showed no interest in them and actually seemed to avoid them although the body walls of the corpses were pierced so that the body fluid was exuding.

Of the Collembola which possess a molar plate on the mandible approximately half of the individuals of each species had no visible gut contents ; the gut of these individuals may be empty or may contain material which is not visible in lactic acid. For the purpose of this survey, only identifiable gut contents were considered and it is hoped to study the specimens without visible gut contents at a later date.

(b) *Collembola with visible gut contents*

The gut contents examined contained the following identifiable materials :

Fungal hyphae and spores.
Cellulose—stomata guard cells and phloem elements.
Lignin—tracheids with scalariform thickening and bordered pits, stone cells.
Mineral particles
Testate Amoebae
Earthworm setae
Collembolan scales.

Fungal hyphae and spores made up the majority of the recognisable material, higher plant tissue was present in small amounts and mineral particles were common. The remainder of the visible gut contents was amorphous debris which varied considerably in amount, but generally represented a larger proportion in the smaller animals.

Description of materials in the gut

Fungi. The hyphae and spores were almost entirely those of the Fungi Imperfecti of the family Dematiaceae, the hyphae of which are dark brown in colour and septate ; they are common soil forms, see Forsslund (1939). The gut contents of a specimen of *Isotomurus plumosus* Bagnall stained with cotton blue in lactophenol showed that one of the hyphae had definite cell contents.

Higher plant remains. The majority of higher plant remains were in the form of pieces of yellow or brown tissue ; in the larger Collembola a small proportion of this was recognisable as tracheids or the guard cells of stomata. It is probable that the majority of the structureless material was of mesophyll

origin since it resembled the decaying mesophyll seen in sections of Douglas fir litter.

To discover how much of this tissue was lignified some gut contents were stained with phloroglucin and the following results were obtained.

TABLE 1

Results of examination of gut contents in Phlororglucin

Species	Date	Non-lignified (percentage)	Lignified (percentage)
Folsomia 4-oculata (Tullberg)	7.10.55	99·9	trace
Isotomurus plumosus Bagnall	7.10.55	99·9	trace
Tullbergia callipygos Borner	7.10.55	100	0
Isotoma notabilis Schaffer	4.5.55	90	10

The above table shows that most of the higher plant tissue in the collembolan gut was non-lignified. This agrees with Handley (1954) who found that in "Mor" soils lignified tissue persists for a very short time in the decaying leaf litter. As there was no ground flora in the study area the higher plant remains were entirely derived from the Douglas fir.

Mineral particles. These occurred in the form of irregular colourless particles.

(c) *Quantitative estimates of the gut contents*

The two important recognisable materials occurring in the gut of most Collembola examined were Fungi and higher plant remains. To assess the role played by Collembola in the breakdown of plant litter a quantitative survey of the gut contents was made with reference to these two foods.

Because of the difficulty of making an even squash of the gut contents on a slide, due to the small size of the animals, any method of assessing the proportions of the different substances present could only be approximate. The method used was simple ; the gut contents were examined microscopically and the total identifiable material was given the value 10. This material was divided into three groups, Fungi, higher plant remains and mineral particles and these were given values in accordance with the proportion in which they were present. (See Tables 2 and 3).

Usually a single estimate was made for the gut contents of a number of specimens but where these differed considerably those of different individuals were assessed separately, e.g. *Folsomia 4-oculata* on 7.7.55 where some specimens had been feeding on Fungi and others on higher plant tissue.

Unidentifiable material. It is extremely difficult to estimate the proportion of unidentifiable material, which usually has the appearance of fine debris. In larger forms such as *Isotomurus plumosus* and *Tomocerus longicornis* there is very little unidentifiable material and the gut is often crammed with fungal hyphae, whereas in a small form, like *Tullbergia krausbaueri* or *Megalothorax*

TABLE 2

Analysis of gut contents of various species of Collembola

Date	Species	Number of specimens	Identifiable material in gut				
			Fungi	Lignin or Cellulose	Mineral particles	Fungi	
						Hyphae	Spores
7.7.55	*Isotoma viridis* Bourlet	8	9·5	0·5	+	7	2·5
,,	*Isotoma notabilis* Schaffer	20	10	tr	tr	8	2
,,	*Isotomiella minor* (Schaffer)	1	9	1	+	8·5	0·5
,,	*Isotomurus plumosus* Bagnall	35	10	tr	+	9·5	0·5
,,	*Folsomia quadrioculata* (Tullberg)	1	1	9	tr	0·75	0·25
		4	9	1	tr	7	2
,,	*Lepidocyrtus lanuginosus* (Gmelin)	10	10	tr	+	8	2
,,	*Tomocerus minor* (Lubbock)	2	9·5	0·5	+	6·5	3
,,	*T. longicornis* (Muller)	10	9·5	0·5	+	9·0	0·5
,,	*Dicyrtomina minuta* (Fabricius)	3	9	1	tr	8	1
,,	*Sminthurides pumilis* (Krausbauer)	10	10	tr	tr	8·5	1·5
,,	*Sminthurinus aureus* (Lubbock)	4	10	+	tr	9	1
1.2.55	*Tullbergia krausbaueri* Borner	10	9	1	0	8	1
7.7.55	*Tullbergia callipygos* Borner	5	9	1	tr	6	3
		5	1	9	tr	0·5	0·5

NOTE. Total identifiable material = 10.
'tr' = present in a small but constant proportion.
+ = present in very small quantities.

minimus Willem most of the material is fine debris with scattered pieces of fungal hyphae ; thus in both the above types Fungi may be given the value 10 but the food is clearly very different. This is a drawback of the method described but it can generally be assumed that the smaller the species the larger will be the proportion of unidentifiable material. These small species are detritus feeding and are probably actually coprophagous or feeding upon the humus itself.

Table 2 shows an analysis of the gut contents of thirteen species most of which were collected on the same date. It is clear from this table that Fungi are the most important item in the diet of the Collembola. There is very little consistent difference between the diets of different species and sometimes there are remarkable variations within one species collected on a single occasion; *Tullbergia callipygos* and *Folsomia 4-oculata*, for example, show some specimens feeding mainly upon the litter and others upon fungal hyphae.

It was thought possible that the diet of Collembola might vary throughout the year and that perhaps Fungi might be more plentiful at one particular time of the year. The results of an investigation of the feeding habits at different times of the year are shown in Table 3. If the species are considered one by one there can be seen to be some variations between specimens collected on different dates.

Considering the three main collecting dates the results are as follows :

1. 13*th December* 1954. *Isotomurus plumosus* and *Isotoma notabilis* were feeding on the Fungi whereas *I. viridis* and *Tomocerus minor* were feeding predominantly on the litter.

2. 4*th May* 1955. All Collembola examined were feeding on both litter and Fungi in approximately equal quantities, except *Isotomiella minor* which was feeding mainly upon litter and *Istoma notabilis* feeding on Fungi.

3. 7*th July* 1955. All species were feeding upon Fungi although one specimen of *Folsomia 4-oculata* was feeding upon higher plant tissue.

These results show that the proportion of Douglas fir tissue consumed varies between samples taken at different times of the year and, unless this effect is due to the sampling of different microhabitats, there is definite evidence of seasonal variations in diet.

(d) *A comparison of the microscopical structure of the humus with collembolan gut contents*

Humus was mounted in lactic acid and examined microscopically, the following items were identified :

Douglas fir remains, both large and fragmentary, stone cells, tracheids, etc.
Fungal hyphae and spores—mostly Dematiaceae.
Mineral particles.
Testate Amoebae.
Nematodes.
Insect remains—including a collembolan leg segment.
Mite remains.

TABLE 3

The effect of the time of year on the gut contents of different collembolan species

Species	Date	Number of specimens	Identifiable material in gut.				
			Fungi	Lignin or cellulose	Mineral particles	Fungi	
						Hyphae	Spores
Isotomurus plumosus	13.12.54	5	9	1	tr	8·5	0·5
		20	9·75	0·25	tr	9·5	0·25
	4. 5.55	15	4·75	5	0·25	3·0	1·75
		15	1·5	8·25	0·25	1·0	0·5
	7. 7.55	4	9·5	0·5	tr	9·5	tr
		31	10	tr	0	9·5	0·5
	11.10.55	10	9·75	0·25	tr	9·75	tr
I. plumosus	Average		8	2	tr		
Isotoma notabilis	4. 5.55	5	10	+	tr	9	1
	13.12.54	25	10	tr	tr	9·5	0·5
	7. 7.55	20	10	tr	tr	9	1
I. notabilis	Average		10	tr	tr		
Isotoma viridis	13.12.54	5	3	7	0	3	tr
	7. 7.55	2	9	1	0	8·5	0·5
		6	10	tr	0	9	1
I. viridis	Average		7	3	0		
Tomocerus minor	13.12.54	3	3	7	+	2	1
	4. 5.55	1	4	5	1	2	2
	7. 7.55	2	9·5	0·5	+	6·5	3·0
T. minor	Average		5	5	+		
Isotomiella minor	4. 5.55	5	3	7	tr	1·5	1·5
	7. 7.55	1	9	1	+	8·5	0·5
I. minor	Average		4	6	tr		
Folsomia 4-oculata	4. 5.55	11	5	5	+	2·5	2·5
	7. 7.55	1	1	9	tr	0·75	0·25
		4	9	1	tr	7	2
F. 4-oculata	Average		6	4	+		

NOTE. Total identifiable material = 10.
'tr'—present in a small, but constant, proportion.

The brown Fungi were very common and most of the larger litter remains were covered with them. It was difficult to compare the humus with collembolan gut contents because of the very large particle size of the former.

Some sections of humus from Waen Wen were prepared for me by Miss S. Hepple (formerly of Department of Botany, Liverpool University) using the technique described by Burges & Hepple (1956). These sections showed that much of the humus consisted of fine amorphous debris which bore a strong resemblance to that found in the gut of the small Collembola. The humus appeared to contain a smaller proportion of fragments of fungal hyphae than did the collembolan gut debris ; it is therefore possible that the small Collembola feed upon the faeces of mycetophagous animals or that they feed upon living Fungi in addition to the fragments occurring in the humus.

(e) *Studies on the food of Tomocerus longicornis* (Muller)

The main object of these studies was to discover whether viable Fungi can pass through the collembolan gut. *Tomocerus longicornis* was selected because it is typical of the larger Collembola in its feeding habits ; it is also large and easily recognised and can therefore be collected with a " pooter ".

Experiment 1

On 6th October 1955 six specimens of *T. longicornis* were collected from the experimental wood and placed in a sterile test tube containing damp filter paper ; they were left overnight and removed the next morning. On 8th October the faeces in the tube were washed to the bottom with sterile distilled water and a suspension made of them. The suspension was taken up in a sterile eye dropper and expelled into a petri dish containing Soil agar.

By 10th October several bacterial colonies had developed on the plate and could be seen to follow the injection lines on the agar ; a week later fungal colonies had also developed. Some of the faeces remaining in the tube were examined microscopically and fungal spores predominated, about 70 per cent of them being thin walled spores; the thick walled brown spores of the Dematiaceae were poorly represented. The results were : Fungi 10, Higher Plant tissues " trace " and mineral particles " + " (of the Fungi, spores=10 and hyphae=" trace "). These results were not typical of this species and therefore a second experiment was carried out.

Experiment 2

Specimens of *T. longicornis* were collected from Waen Wen on 11th October 1955, some were killed for examination of the gut contents and the rest were placed in two sterile test tubes ; one tube contained moist filter paper and thirteen Collembola and the other a drop of distilled water and ten Collembola. The animals were left in the tubes over night and recovered the next day. Some of the faeces in the first tube were picked off with a platinum wire and smeared on peptone agar in three petri dishes. A suspension was made in the second tube and this was smeared on peptone agar in three other petri dishes using a platinum loop.

By 18th October five of the six petri dishes showed fungal colonies growing along the innoculation lines, showing that viable Fungi can pass through the

NOTE. Total identifiable material = 10.
't' = present in a small, but constant, proportion.

collembolan gut. It is possible that fungal spores were carried by the Collembola on their bodies and accidentally contaminated the faeces but this is unlikely in view of the consistent results.

The gut contents of some of the Collembola collected on 11th October were examined and, in addition, two slides were made of the faeces remaining in the sterile tubes ; the results are shown in Table 4.

TABLE 4

Analysis of faeces and gut contents of *T. longicornis* collected on 11th October 1955

	Fungi	Higher plant tissue	Mineral particles	Fungi	
				Hyphae	Spores
Gut contents of 15 specimens	9·5	0·5	+	9	0·5
Faeces Slide 1	9·5	0·5	+	8·5	1
Faeces Slide 2	9	1	+	7	2

The faeces squashes and the gut contents agree very well although the former contained a rather higher proportion of fungal spores.

The fungal colonies were sub-cultured on to weak Sabauraud agar (30 gms. per litre) and although many of the Fungi isolated were common moulds (whose spores occur in large numbers in the soil) two colonies were isolated which had dark brown septate hyphae. These cultures were identified as members of the family Dematiaceae and resembled the hyphae found in the collembolan gut and faeces.

From this it seems probable that viable Fungi can pass through the gut of Collembola and that as Collembola appear to feed mostly upon Fungi they may play an important role in the dispersal of the soil Fungi.

DISCUSSION

This survey of the feeding habits of Collembola has shown that in Waen Wen the larger Collembola feed mainly on soil Fungi and although large amounts of decaying higher plant material were occasionally found in the gut this cannot be said to be their main diet. In view of the fact that the gut is usually packed with fungal hyphae there is strong evidence that Collembola are selective feeders and seek out fungal colonies in the soil. In regions where Fungi are not available they may turn to decaying litter as a second choice. It is probable that decaying litter is only palatable in a certain state of decay, and it may be that they prefer this but that the supply is limited. It is likely that Collembola play an important part in the dispersal of soil Fungi, which appear to pass through their gut unharmed.

The smaller Collembola feed on the humus and probably also on arthropod faeces in the litter layer. Much of the material in the gut is amorphous detritus but the high proportion of fungal fragments suggests that they may be feeding on the faeces of mycetophagous animals. It is difficult to know

whether they feed directly on living fungi or merely the dead remains in humus and faeces. The fungal walls are very resistant to digestion (as shown by the examination of collembolan faeces) and are then probably highly resistant to decay.

There appear to be no marked differences in feeding habits between the solid feeding species, although individual variations may be considerable. It is interesting to note that Forsslund (1939) found large quantities of fungal hyphae in the gut of some oribatid mites.

It is probable that Collembola feed on different foods at different times of the year and this may be related either to a scarcity of Fungi or to an abundance of palatable litter remains. Until more is known of the palatability of litter and the preference of the Collembola, little more can be said.

It is not possible at this stage to make a true assessment of the role of Collembola in the breakdown of plant litter and in the general ecology of the soil. Their numbers and activity suggest that they may be of considerable importance. The direct value of Collembola as litter feeders is probably small judging solely from their gut contents. On the other hand, they must play some part, both chemical and physical, in the breakdown of humus. Schaller (1949) has shown that they feed on the faeces of larger arthropods thus comminuting these large particles and changing them chemically, they have also been observed by the author eating their own faeces and those of other Collembola. The larger Collembola, in feeding on the Fungi growing in the litter play an indirect part in converting the litter to humus. It may be assumed that they do not completely destroy fungal colonies but merely browse on them and therefore form a significant link in the chain of litter breakdown. Their role as dispersal agents for soil fungi is impossible to assess but it may well be of great importance as soil fungi produce numerous spores which must rely largely on animals for transport.

SUMMARY

An examination of the visible gut contents of Collembola showed that the larger species feed mainly on soil Fungi, whereas the smaller forms appear to feed directly on the humus. There seemed to be no consistent differences in the feeding habits of species of similar size but there was some evidence of seasonal variations in the amount of Douglas fir litter consumed by Collembola.

Experiments with *Tomocerus longicornis* indicated that viable soil Fungi pass through the collembolan gut ; it is therefore probable that they play an important role in the dispersal of soil Fungi.

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THE RESPONSE TO LIGHT IN *ICHTHYOPHIS* (AMPHIBIA-APODA) FROM CEYLON

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(With 2 plates)

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INTRODUCTION

Studies on the response to light in vertebrates whose eyes have become reduced or lost have revealed a number of different types of dermal photosensitivity. Lampreys, both as larvae and adults, possess photo-receptors on the tail which are innervated through the posterior fibres of the lateralis (Young, 1935). The hagfish, *Myxine glutinosa*, responds to general illumination, and to local illumination on both head and tail, but here the spinal nerves link the photo-receptors to the central nervous system (Newth & Ross, 1955). The cavernicolous urodele, *Proteus anguinus*, is sensitive to light directed both on the head and on the tail, and the eyes are not necessary for the head response (Hawes, 1945). There is also an extensive older literature on the light responses of tadpoles (Laurens, 1914 ; Obreshkove, 1921), blind fishes (Payne, 1907 ; Parker, 1909), frogs (Parker, 1903), and blind and decapitated salamanders (Eycleshymer, 1908 ; Sayle, 1916 ; Uhlenhuth, 1921). All these papers produce evidence of dermal photo-sensitivity in these animals without throwing much light on its physiological properties or its neurological basis. It would seem that amongst the Anamnia at least, the vertebrate skin has a general capacity to develop photosensitivity in those cases where the eyes are absent or defective. There is a need, however, to examine some of these cases again and to extend the study to include other examples so that the precise nature and properties of the adaptation will be known over a wide range of lower vertebrates.

Amongst the eyeless vertebrates whose light responses have not been investigated is the important and ancient amphibian order Apoda, burrowing legless forms, circumtropical in distribution. An opportunity to study these animals came when a few specimens of the Oriental species, *Ichthyophis glutinosus*, arrived in this laboratory from Ceylon in 1955. It was found that *Ichthyophis* is normally inactive in the dark, only moving about occasionally in search of food. However, if a relaxed quiescent *Ichthyophis* is illuminated suddenly, it is soon roused and may remain active for some time, though after a few minutes in the dark the animal generally becomes quite still again. It was clear that *Ichthyophis* is sensitive to light and it seemed likely that since the response took the form of a distinct arousal from rest, it might lend itself to the quantitative and physiological studies required to reveal the nature of its photo-receptive sense.

Of the considerable number of morphological and systematic works on *Ichthyophis* and the Apoda generally, two papers are particularly relevant to this investigation. Kuhlenbeck (1922) gave a general description of the anatomy and histology of the brains of *Ichthyophis glutinosus*, *Hypogeophis rostratus* and *Siphonops annulatus* in which he reported finding no trace of an optic chiasma and in the hypothalamus detected only an extremely thin thread-like optic nerve. Engelhardt (1924) presented a very full account of the morphology of the eye and the tentacle of *Ichthyophis glutinosus* and structures associated with them in the orbital region. He described the optic nerves as rudimentary threadlets, only 8 μ thick near the brain where they form no chiasma. The nerve passes through the large orbital gland and becomes somewhat thicker before entering the eye where it divides into three branches on the retina.

The eye is very rudimentary and probably represents merely a survival into adult life of the functional eye of the larva before metamorphosis. The lens and the iris are just visible externally through the covering layer of skin. This skin is thin compared with that on the rest of the head and shows certain histological peculiarities but it in no way corresponds to a true cornea, though the inner layer is sometimes given that name. The lens is tightly applied to the inside of this covering skin, the pupil is very wide, equalling the diameter of the lens, and there is no ciliary body. Sclerotic and choroid coats are clearly visible and the retina shows a considerable degree of histological differentiation. Engelhardt distinguishes seven layers of cells with a basal layer of rods lying innermost next to the pigment layer. But this retina is very thick compared with the eye itself. The dimensions taken from the figures in Engelhardt's paper give the eye a diameter of about 0·5 mm. and the retina a thickness of about 0·1 mm.

Although it was clear from the work of Engelhardt and others that the eyes of *Ichthyophis* are very defective and certainly cannot form images, it could not be assumed that they played no part in the animal's response to light. Therefore, one of the objects of the investigation was to find out experimentally what part, if any, the eyes play in the response to light in *Ichthyophis*. This could be done, however, only when the response had been examined quantitatively. In the first instance it was necessary to find out

if reaction time could be used in these animals as a measure of photo-sensory activity. To do this it was necessary to measure the reaction times of *Ichthyophis* under various conditions of illumination, and this was regarded as the necessary first stage of the investigation. Subsequently, local illumination was used to see what areas of the animal are sensitive to light and finally, the effects of certain surgical operations on the character and timing of the response were investigated.

MATERIALS AND METHODS

The fourteen animals used in the experiments were all obtained from Ceylon through the great kindness of Major W. W. A. Phillips, and they came to London in three groups between February, 1955 and June, 1956. Thirteen of these were recognisably *Ichthyophis glutinosus*. The remaining one corresponded more closely to descriptions of *I. monochrous* (Deraniyagala, 1933 ; Kirtisinghe, 1957), a smaller species lacking the conspicuous lateral yellow stripe found in *I. glutinosus*. In the laboratory they were kept in the dark in separate containers each with enough soil to cover the animal. They were kept moist and fed earthworms once or twice a week. At first, glass containers were used but these were replaced by boxes made of wood and hardboard after some animals had sickened and died, apparently due to unsatisfactory conditions in the glass jars.

All tests were carried out in standard boxes, the floors of which were covered with soil to a depth of about $\frac{1}{4}$ in. One wall of the box was made of transparent plastic as an observation window. In another box of exactly the same dimensions the floor was fitted with twenty 6 V tungsten lamps connected to a mains source through a 12 V transformer. The wiring was arranged so that the lights could be switched on in pairs. By turning this box upside down and resting it on top of the box containing the animal, with a plate of ground glass in between, the animal could be exposed to general illumination of a fairly uniform type. By switching more lights on or off the intensity of illumination could be altered fairly easily, the lights being distributed in the box in such a way that all parts of the box were close to sources of illumination whatever the group of lights in use. Table 1 shows the range of intensities obtained by this simple apparatus, which by inserting additional screening materials, such as tracing paper or more sheets of ground glass, between the two boxes, can be modified to produce intensities as low as one requires. This Table shows, however, that the aim of uniform illumination throughout the observation box was only partially fulfilled by this arrangement. In every case the illumination was more intense at the centre of the box, and at the lowest intensities the centre was almost three times as bright as the far corners. However, the equipment provided an intensity range of about 80 : 1, and for the experiments envisaged these intensity differences were sufficiently sharp to test the relation between the reaction times of *Ichthyophis* and the intensity of illumination.

For work on local illumination, rectangular rods of transparent plastic were used, these being fixed in a light-proof cylinder containing a light source.

By mounting this equipment at the end of a ball-jointed arm and fixing on a rack and pinion stand, the illuminating rod could be brought close up to the surface of the animal with the very minimum of disturbance. The rods deliver a cold light which can be directed on to the skin and the size of the illuminated patch can be varied by using rods of different dimensions.

Animals being used for experiments were continuously illuminated with 3–6 V lamps painted red, one in the centre and one at each of the ends of the observation box. This amount of "safe" illumination was essential in order to be able to see the condition of the animal before exposure to experimental illumination, and in order to follow what happened after the light was switched off. It was also essential for bringing the rods into position for tests on local illumination. Even with this amount of red light it was not always easy to see this dark-coloured animal against the dark layer of soil which formed the background.

TABLE 1

Range of intensities of illumination (foot candles) in experimental box.

No. of lamps	Intensity of general illumination (f.c.)		
	Centre of box	Corners of box	Mean
20	90	68	79
10	60	45	53
2	16	12	14
20*	9·3	4·8	7
2*	1·5	0·5	1

* With eight layers of tracing paper interposed between light sources and observation box.

No special precautions were taken to exclude temperature effects in the experiments with general illumination. At intensities up to and including 14 f.c., such effects were probably unimportant. Illumination at 14 f.c. for 60 sec., the longest exposure employed, caused a rise of less than 0·1° C. on the floor of the box. At the highest intensity used, 79 f.c., illumination for 60 sec. was accompanied by a rise of temperature of 0·4° C. on the floor of the box. Consequently possible heating effects of the illumination will have to be borne in mind in dealing with the results at this intensity. Fortunately, except in some experiments on operated animals, the duration of illumination at these higher intensities seldom exceeded a few seconds during which the rise in temperature could hardly be more than a few-hundredths of a degree. Observations were spaced sufficiently far apart (at least 30 min.) to avoid the possibility of a steady rise in temperature from repeated illumination during a series of experiments. The continuous illumination of the box by the red lights provided a source of heat that maintained the animals under observation at a fairly high, but uniform, temperature within the range 21°–23° C.

RESULTS

Responses to general illumination

There are certain variable features in the response of *Ichthyophis* to light that make it less satisfactory to study than the responses which occur in lampreys, hagfish and tadpoles. There are circumstances, not easily defined, when the animal fails to respond at all, even to intensities which normally produce a clear-cut response. Animals that have recently been fed, or which have just stopped moving about after a period of activity, show this tendency, but often there is no such obvious explanation. Thus at all the intensities employed there were a certain number of " no responses ".

Moreover, the response did not always take the same form in different animals. In six of the animals tested, the only response observed was a general coiling and locomotry movement. This began with signs of stirring, as from a deep sleep. First the head was raised and this was usually followed by gentle swaying movements which moved the anterior part of the body from side to side, and finally the entire body became involved in the movement, which after a period of twisting and writhing developed into locomotion of a typical serpentine type sometimes with intermittent attempts to burrow. Photographs of an *Ichthyophis* at rest and engaged in non-locomotory writhing are shown in Plate 1 along with a photograph of the animal ingesting an earthworm. This pattern was repeated with individual variations many times, and if the animal responded at all to light, this type of response generally appeared at some stage in the period of observation. Yet the time elapsing between the beginning of illumination and the beginning of this activity proved to be extremely variable. Preliminary experiments showed that even with intensity kept constant, as with all twenty lamps turned on, this response might begin at any time from a couple of seconds up to a minute after switching on the light. Consequently, attempts to obtain satisfactory intensity reaction time relationships from this response seemed likely to fail completely.

In a second group of six animals the general response was not the only response to light. In these, illuminating a quiescent animal caused a pronounced twitch or shudder within a few seconds of the beginning of illumination. This twitch response was sometimes followed almost at once by the usual general stirring and locomotory movement described above. At other times, an interval of anything from a few seconds to more than a minute might separate the two responses, and sometimes the twitch response alone occurred. But the time elapsing between the beginning of illumination and the twitch response showed a high measure of constancy with constant intensity and it seemed, therefore, that this response might give reliable quantitative data on photo-receptive processes in *Ichthyophis*. Work on this aspect of the problem was concentrated, as a result, on those animals which showed a clear twitch or shudder and in fact, most of the data were obtained from five animals. One animal in particular (it happened to be the specimen suspected of being *I. monochrous*) was studied intensively and from it came all the data on the relation between the duration of illumination and reaction time.

TABLE 2

Reaction times (twitch responses) of five *Ichthyophis* at four intensities (foot candles) in sec. Values are means of ten readings except those with asterisks which are means of five. Blank spaces indicate failures to obtain twitch responses.

	Intensity (f.c.)			
Animal	79	14	7	1
A1	1·54 sec.	2·30	2·53	10·7
A2	1·57	1·85	2·11	2·74
B1	2·44	3·82	7·90	—
C1	2·27	7·39	8·70*	—
C2	2·84	16·8*	—	—

TABLE 2a

Results of individual tests on Animal A2 with general illumination at four intensities showing reaction times of twitch response (TR) and general response (GR) whenever one or both responses occurred following illumination. Identical figures for TR and GR with the sign "+" on the latter indicates that the twitch response led directly, with no detectable pause, to a general response. Spaces marked "—" indicate failures to obtain either a twitch or a general response in a given test. Mean reaction times (disregarding "no responses") are given for the ten twitch responses obtained at each intensity but the range of reaction times for general responses is too wide for means to have any value as a measure.

Intensity (f.c.)							
79		14		7		1	
TR	GR	TR	GR	TR	GR	TR	GR
1·5	—	1·8	—	2·0	2·0+	2·6	2·6+
1·5	—	1·8	—	2·8	5·0	3·0	20·0
1·6	—	1·8	10·0	2·0	—	—	9·0
1·6	1·6+	1·6	—	—	95·0	2·0	—
1·6	1·6+	2·2	2·2+	2·0	12·0	—	50·0
—	13·7	2·0	2·0+	1·8	10·5	3·5	45·0
1·6	1·6+	1·6	1·6+	—	29·0	2·4	—
1·6	1·6+	—	15·8	2·0	22·0	2·2	—
1·5	1·5+	2·0	—	2·2	—	—	21·0
1·6	1·6+	1·5	—	1·8	1·8+	—	37·0
1·6	1·6+	—	8·9	2·5	2·5+	2·6	—
		—	12·2	2·0	15·0	2·6	—
		2·2	—			3·5	3·5+
						—	13·0
						3·0	17·0
Means							
1·57		1·85		2·11		2·74	

Table 2 gives the mean reaction times of the twitch response for the five animals separately and Table 2a details on the performance of the more intensively studied Animal A2, at four different intensities. The individual animals were clearly so different in their sensitivity to light that it would be misleading to average their reaction times, yet each animal was fairly consistent in its own performance, surprisingly so when one considers the variables in the experimental situation.

In the above tests the animal was illuminated until it began to move about actively, or for one minute if it remained still. Table 3 shows the results obtained when, at two different intensities, Animal A2 was illuminated for steadily decreasing periods below the reaction times. One of the elements in this situation is an increase in the number of " no response " results as the duration of the illumination becomes shorter. At both intensities, however, before the point is reached at which all twitch responses cease, one sees twitch responses whose reaction times are longer than normal. This demonstrates that the reaction time of the twitch response in *Ichthyophis* is made up of the two components discovered by Hecht (1918) in his classical experiments on *Ciona* : (1) sensitization time—the period of illumination necessary to elicit a response in minimum time, the period during which the presumed photochemical reactions are taking place ; (2) the latent period, during which illumination is not necessary, when it is presumed that the secondary processes

TABLE 3

Numbers of twitch responses with mean reaction times and numbers of general responses in *Ichthyophis* A2 when period of illumination is progressively shortened at two intensities. Figures in parentheses are numbers of general responses not preceded by twitch response, i.e., nos. of observations in which general response was the only response.

Illumination		No. of observations	No. of twitch responses	Mean reaction times sec.	No. of general responses
Intensity	Duration				
f.c.	sec.				
79	0·50	10	10	1·52	4 (0)
	0·40	10	7	1·43	6 (1)
	0·30	10	3	1·60	5 (4)
	0·22	10	5	1·50	4 (1)
	0·17	15	8	1·44	4 (3)
	0·10	15	4	7·35	10 (8)
14	0·70	19	10	1·80	11 (6)
	0·63	10	4	1·50	4 (2)
	0·50	31	10	4·14	16 (9)
	0·30	15	0	—	12 (12)

are taking place by which the photochemical events give rise to neuromuscular activities. For the intensities used in these tests we get estimates of $<0{\cdot}17$ and $>0{\cdot}10$ sec. for the sensitisation time at an intensity of 79 foot candles,

and <0·63 and >0·50 sec. at 14 foot candles, and corresponding figures 1·40–1·47 sec. and 1·22–1·35 sec. for the latent period, using the values in Table 2 as the standard minimal reaction times at these intensities. The relationship between the sensitisation times at the two intensities is not far removed from the expectations of a purely photochemical event following the Bunsen-Roscoe Law since a five- to six-fold increase in intensity gives a sensitisation time four- to five-times as brief. Indeed, the whole picture emerging from these intensity/duration/reaction time relationships makes it plain that the twitch response of *Ichthyophis* has much in common with a response to light of the classical Hecht type. Having established that point it was not necessary to pursue this aspect of the problem further.

Although the time elapsing between the beginning of illumination and the first movements of the general response are extremely variable and seemed quite unsatisfactory as a photo-receptive reaction time (cf. Table 2a) the general response is not without interest. Indeed, for the animal, it is probably the more important of the two responses. In about half the animals studied, the general response was the only response to light and if the data adduced above are to have any bearing on the life of *Ichthyophis*, we must establish in what relationship the two responses stand to each other. A scrutiny of all the results obtained from the experiments with general illumination produced some important additional data.

Table 4 sets out the number of responses, both twitches and general responses, at four different intensities in the five animals that were intensively studied. The number of twitch responses declines steadily and it may be presumed that at intensities a little below 1 foot candle, this response must disappear. In fact all the twitch responses obtained at this lowest intensity were from two out of five animals. The number of general responses, however,

TABLE 4

Summary of data on numbers of responses to light in five *Ichthyophis* at four intensities. Each response (Col. 3) might take the form of a twitch alone (parentheses Col. 4), a general response alone (parentheses Col. 5) or a twitch followed by a general response almost at once (Col. 6) or after some delay.

Intensity f.c.	No. of observations	No. of responses	No. of twitch responses	No. of general responses	No. of general responses from twitches
79	67	62	45 (15)	47 (17)	19
14	65	62	45 (9)	53 (17)	14
7	66	51	29 (7)	44 (22)	8
1	64	40	15 (7)	33 (25)	2

declines much more slowly and it seems likely that they would continue to occur at much lower intensities of illumination, but with such long reaction times that they would be overlooked in experiments like these.

A similar situation is seen in the results with lighting of limited duration on Animal A2. By progressively reducing the exposure to light, twitch

responses become more infrequent and can disappear altogether (Table 3). But even when this happens the general response still occurs in a large number of cases and it would seem that this response can be released, though only after prolonged decay, by much shorter and weaker exposures than the twitch response requires.

The information obtained from the tests so far described allows one to make certain inferences about the response of *Ichthyophis* to light. The basic and essential response in the adaptational sense is the general response by which the animal rouses itself and eventually moves about. Although it is erratic in its appearance and highly variable as a timed reaction, it occurs in all the animals studied and was evoked by the entire range of intensities and durations of illumination examined. But it is obviously a complex motor pattern elicited at the end of at least two and perhaps several stages of neurosensory activity. This is why it is so unpredictable and so variable when compared with the almost automatic light responses with which Hecht worked and which are seen also in lampreys (Young, 1935) and to some extent also in *Myxine* (Newth & Ross, 1955).

The twitch response, on the other hand, seems to be more in the nature of a shock effect of no particular significance to the animal as an adaptive behaviour pattern. Its importance in the physiological sense arises from the fact that it lifts the curtain on the first stage of the response to *Ichthyophis* and makes it possible to subject it to experimental analysis. It shows that illumination of certain intensities and durations can elicit a response that has some of the characteristics of a simple photo-sensory process. It reveals how long it takes an *Ichthyophis* to translate light energy falling upon it into nervous impulses leading to motor activity when presumably there are minimal barriers to conduction in the central nervous system. The fact that only some of the animals showed the twitch response does not diminish its value here as the outward sign of the first stage of the general response to light. It occurs often enough and consistently enough for one to infer that the processes of peripheral reception leading to central excitation, revealed by the twitch, have the same characteristics in all *Ichthyophis* even when there is no twitch or shock response to mark their completion.

What happens after this first stage is completed can only be guessed at, but presumably, the motor centre from which general locomotory responses arise begins to receive impulses from the photo-receptive centre. But the motor centre, possessing a great variety of connections with other sensory centres and subject to facilitation and inhibition in its relations with any one of them, may or may not be excited by the impulses arriving from the photo-receptive centre, or may be excited only after considerable delay. That the length of this delay depends to some extent on the intensity and duration of the illumination is made clear in the results presented in Tables 3 and 4, especially in Column 6 of the latter where it is shown that at high intensities, a large proportion of the general responses follow the twitch response almost at once,. that is, with almost no delay. But the problems which arise in trying to be more precise on such questions would be formidable enough in an

animal whose nervous system and behaviour were well worked out, and since the general situation is clear enough there is no need to discuss the matter further.

Responses to local illumination

The surfaces of six *Ichthyophis* were explored by directing upon them light from plastic rods as described above. The two sizes of rods used produced illuminated patches 20×8 mm. and 8×8 mm. in extent. The local intensity of the illumination obtained in this way was of the order of 75–80 foot candles when the rods were at a distance of $\frac{1}{2}$ in. from the animal, almost exactly the same as that obtained with the highest intensities of general illumination employed in the earlier tests.

The heads and tails of the animals were tested at least ten times each with each size of rod. The mid-body region was also tested at least ten times with the larger rod on four of the animals, and from time to time, other parts of the body were explored but not subjected to standard tests. The usual routine for testing was to illuminate the animal until movement occurred or, in the absence of movement, for one minute with the larger, and for ten minutes with the smaller rod. Various methods were used to screen head from tail when one or other was being illuminated but the most satisfactory method proved to be to have small heaps or mounds of soil in the box and to carry out tests only when the head was below the mound on one side and the tail below it on the other.

The collected results of all the tests with local illumination on head and tail were quite consistent in demonstrating sensitivity to light in those regions. In over 140 tests on the tails and 125 on the heads, there were only eight occasions when responses failed to occur, a lower proportion of failures than with general illumination in the same animals. Twitch responses, however, were almost never seen, even in animals which regularly gave this response to general illumination. The precise form of the response to local illumination was highly variable. Head illumination generally gave rise to stirring movements of the head leading to its removal from the light, and as a rule, the rest of the body later became active. Caudal illumination almost always produced a definite local movement which removed the tail from the light and in the majority of tests, after a pause of from 10 to 30 seconds, the anterior part of the body then began to show typical pre-locomotory stirring movements often developing into locomotion. There can be no doubt, therefore, that both the head and tail of *Ichthyophis* are sensitive to light.

Sensitivity to light in other regions of *Ichthyophis* is more problematical. Local illumination of the mid-body region was virtually ineffective in two animals, produced a few responses in one other, and in the remaining animal gave responses every time, but only after long delays. Illumination between the mid-body region and head or tail produced the same result, occasional responses after long exposures.

Table 5 sets out the reaction times, measured to the first visible movement, with local illumination of head, tail and mid-body regions. It is not surprising that the figures in some animals showed a wide variation indicated by the

TABLE 5

Reaction times of responses of six *Ichthyophis* to local illumination on head, tail and mid-body using rods of transparent plastic which conduct light from a remote source and illuminate patches 20×8 and 8×8 mm. in extent. All values are means of reaction times for ten responses except those marked [1], [2], and [3] which are means of 23, 13 and 14 responses respectively. In the last column, the reaction times are based on the number of responses in ten observations and these figures are given in parentheses.

Animal	Reaction times (sec.) with local illumination of different regions						
	Head			Tail			Mid-body.
	20×8 mm.	St. Dev.	8×8 mm.	20×8 mm.	St. Dev.	8×8 mm.	20×8 mm.
A2	9·1 sec.	±4·4 sec.	21·7 sec.	4·3[1] sec.	± 2·7 sec.	6·2 sec.	29·0 sec. (3)
B3	13·9	±5·3	21·6	5·4	± 2·7	8·2	—
B4	15·1	±9·2	28·3	15·7[2]	±13·3	26·3	10·0 (1)
B5	13·3	±7·4	23·3	15·2	±17·5	29·3	50·7 (10)
B6	7·7	±2·7	10·8	4·0	± 1·4	7·9	49·0 (1)
C2	10·3	±6·4	24·5	2·5[3]	± 1·1	3·0	—

size of the standard deviations, considering the variables in these experiments in which the rods had to be brought into position in semi-darkness. In Animals A2 and C2 in particular, which gave twitch responses with general illumination, the reaction times with caudal illumination were remarkably consistent, and indeed quite close to the twitch reaction times. Perhaps the most interesting fact emerging from these data is that the shortest reaction times were generally those of tail responses and that in four out of six animals studied, the consistently briefer reaction times of the tail suggest that it is much more sensitive to light than the head.

Attempts were made by siting the 8×8 mm. rod in different places on head and tail, and by reducing the illuminated area to 4×4 mm., to find out if there were any particularly sensitive areas of the skin on the head or tail of *Ichthyophis*. By painting small spots on the skin as landmarks it was possible to be fairly certain about the locations of the sites illuminated. The data for three animals (ten readings on each site) indicate that the head is fairly uniformly sensitive. Reaction times are substantially the same whether one illuminates the areas in front of the eyes, between the eyes, laterally directly into the eyes, or immediately behind the eyes. But from 1–2 cm. from the tip of the head, the sensitivity falls off sharply.

On the tail the distribution of sensitivity proved more interesting. To obtain a response it seems to be necessary to illuminate the tail tip. All the results summarised above in Table 5, in which the tail responded almost without fail, were obtained by illuminating patches which included the tail tip. Some later experiments in which the tip was obscured or not lit directly, gave responses in less than 20 per cent of trials (forty-five tests on four animals gave eight responses). Apparently, to obtain a response, it is necessary to illuminate the last few millimetres of the animal.

The results of the tests with local illumination confirm the impression gained from experiments with general illumination that most of the delay in the development of the stirring and locomotory responses is central in origin. The local tail response, like the twitch response described earlier, demonstrates that the intensities of illumination employed here can generate motor activity in the space of about two seconds. The general response following after a much longer period, depends on the fulfilment of something more than purely reflex conditions.

Responses following section of the spinal cord and optic nerve

In view of Young's (1935) demonstration of the role of the lateralis as the peripheral pathway from the caudal photo-receptors to the brain in lampreys, the possibility of a non-spinal route in *Ichthyophis* needed to be investigated by examining the effects of spinal section. It was also important to discover what capacities for independent responses and activity remained in those parts of a spinal animal posterior to the cut. Moreover, although the tests with local illumination did not reveal any special sensitivity in the eye region, and although the eye is known to be almost without functional connections with the brain, more direct experimental evidence was needed on the role of the eye in the head response. Severance of the optic nerves offered the most practical means of obtaining such evidence.

Only four animals were available for the operation. Two of them (B5 and B6) showed no twitch or shock reflex responses but they gave good general responses and their behaviour with local illumination had been carefully studied. The other two animals gave twitch responses but on one of them the data for local illumination were fragmentary. The operations were carried out under ether anaesthesia. The vertebral column is deeply embedded in muscle, and column and cord were broken by a small pair of bone forceps inserted into a deep dorsal incision about one-third of the distance from head to tail. The optic nerve was severed by inserting a sharp scalpel behind and slightly deeper than the eye and moving it a few millimetres forwards and backwards. Such an incision will chiefly affect the orbital gland and the retractor muscle of the tentacle and must certainly cut the optic nerve. Unless it penetrates the maxilla below, it should not sever the branches of the trigeminal on their way to the tentacle and the side of the snout.

The results are summarised in Table 6. They show that only two of the animals ever returned to normal as far as their reaction times were concerned, although all four animals responded to light stimuli within 24 to 36 hours after the operation. The effect of spinal section is to cause the animal to give responses independently in front of and behind the cut. The tail continues to be light sensitive and gives the normal local reflex response which takes it out of the field of any local illumination. To general illumination, however, there is frequently no apparent caudal response. On no occasion was any general coiling or locomotory activity observed behind the cut that could be attributed to illumination. Apparently this can only develop when connections with the brain are intact.

TABLE 6

Reaction times (sec.) of four *Ichthyophis* to general illumination (79 f.c.) and to local illumination (20 × 8 mm. rod) before and after operations in which the spinal cords were transected and the optic nerves severed. After the operations no twitch responses occurred but head and tail respond independently, hence the separate columns in the table. Blank spaces indicate failures to respond to illumination. Values are means of ten observations.

Animal	Before operation				After operation			
	General illumination		Local illumination		General illumination		Local illumination	
	Twitch	Locom'y	Head	Tail	Head	Tail	Head	Tail
B5	—	20·2	13·3	15·2	66·4	40·5	22·1	43·2
B6	—	17·0	7·2	4·0	10·5	—	11·2	4·8
C1	2·27	21·6	9·5	4·8	17·2	6·8	12·2	7·4
C2	2·84	14·7	10·3	2·5	33·0	—	27·3	17·2

Table 6 shows that section of the optic nerve does not abolish the local response of the head to light and in two of the animals the reaction time of this response was not very different from that obtained before the operation. The heads were explored with light from rods giving smaller illuminated patches and this time it was found that responses were obtained easily to light directed on the mid-dorsal area of the head but not to light directly on the eyes. This is no proof that the previous response to light falling on this region originated in the eyes, since the post-operative failure may simply be due to interruption of pathways from the skin caused by the incision. Nevertheless, the general head response does not require the presence of eyes and must be attributed, as expected, to photo-receptors scattered over the entire cephalic surface.

Stimulation of the head in operated animals led to general locomotory activity in all regions anterior to the spinal section. Photographs (d) and (e) in Plate 1 show a spinal animal under general illumination which is showing great activity anteriorly and remaining perfectly still behind the cut. The photographs in Plate 2 show the effect of local illumination on the tail in an operated animal, with the strictly localised movement which takes the tail out of the light within a few seconds but without causing any movement beyond this region.

DISCUSSION

The chief point requiring discussion is the role that the response to light as analysed in these experiments plays in the life of the animal. *Ichthyophis* is a burrowing creature and is normally found only by digging or by being accidentally uncovered. Reports on its normal activities are scanty but it is said to make nocturnal excursions from its burrow in search of food, for mating

and egg-laying. In nature, *Ichthyophis* probably never encounters sudden high intensities of illumination like those employed in these experiments. Consequently, the shock reflex can be regarded as a purely laboratory phenomenon, of no adaptational significance to the animal whatsoever. On the other hand, the general response would bring the animal back under cover whenever general illumination began to increase. The long and variable reaction times of the general response at low intensities of illumination, probably taking several minutes for the intensities occurring in nature, say at the approach of daylight, would fulfil the requirements of the situation admirably, causing it to take cover in a leisurely fashion. The animal can have little or no need for a response to light producing a sudden automatic quick withdrawal. Responses to certain other stimuli are very different. Touching an *Ichthyophis*, or exposing it to strong heat or chemical stimuli, produce almost instantaneous and very vigorous activity. The movements accompanying the ingestion of an earthworm, illustrated in the still photograph (c) in Plate 1, are much more vigorous than movements in response to light. It would be obviously inappropriate for *Ichthyophis* to respond to light in such a manner.

The local sensitivity to light revealed by illumination at the head and the tail also fits the needs of the animal admirably. This sensory equipment will provide the animal with information about the amount of light outside the burrow whenever either the head or tail happens to emerge near the entrance. From the results of the experiments it may be inferred that the local illumination of either extremity will cause the animal to withdraw into the burrow again. This is therefore sensory equipment that has protective and exploratory value in the adaptive sense.

This demonstration of a modest photo-receptive sense in the blind *Ichthyophis* which meets its special needs so well, provides a remarkable example of sensory adaptability. It is an impressive addition also to the list of vertebrates without, or with reduced eyes, whose skins have assumed the special but limited photo-sensory tasks associated with lives spent in dark or underground conditions. It would be interesting to know more about the development of this dermal sensitivity to light and to compare the situation in *Ichthyophis* with that in other members of the Order.

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SUMMARY

1. Specimens of the virtually blind apodan, *Ichthyophis glutinosus*, have been exposed to general and local illumination, their sensitivity to light confirmed, and the character and timing of the resulting movements analysed.

2. To general illumination the animal usually gives a general stirring and locomotory response after a considerable and variable delay.

3. In some animals this general response is preceded by a shock reflex, the twitch response, the reaction times of which are reasonably consistent and show the intensity/duration relationships discovered by Hecht in automatic light reflexes in *Ciona* and other animals.

4. The twitch response gives an estimate of the minimal reaction time and shows that the long delay and variability in the timing of the general locomotory movements are due to central processes which determine the response to light in accordance with the general condition and activity of the animal at the time.

5. *Ichthyophis* is sensitive to light falling anywhere on the head, and on the tail from above. The rest of the body is only slightly sensitive.

6. Local illumination on the head or tail gives rise usually to the general locomotory response, but with caudal illumination a local reflex with a very short reaction time precedes the general response. Judged both from reaction times and from numbers of responses obtained, the tail seems to be more sensitive to light than the head.

7. Operations showed that for a general response to develop from caudal illumination, the spinal cord must be intact, but the local tail reflex is not affected by spinal section.

8. The response of the head to light was not affected by severing the optic nerves.

9. The time relations, sensitivity, general character and spatial distribution of the dermal light sense of *Ichthyophis* are discussed in relation to the mode of life of the animal and it is concluded that in all respects, its properties coincide very remarkably with the animal's needs.

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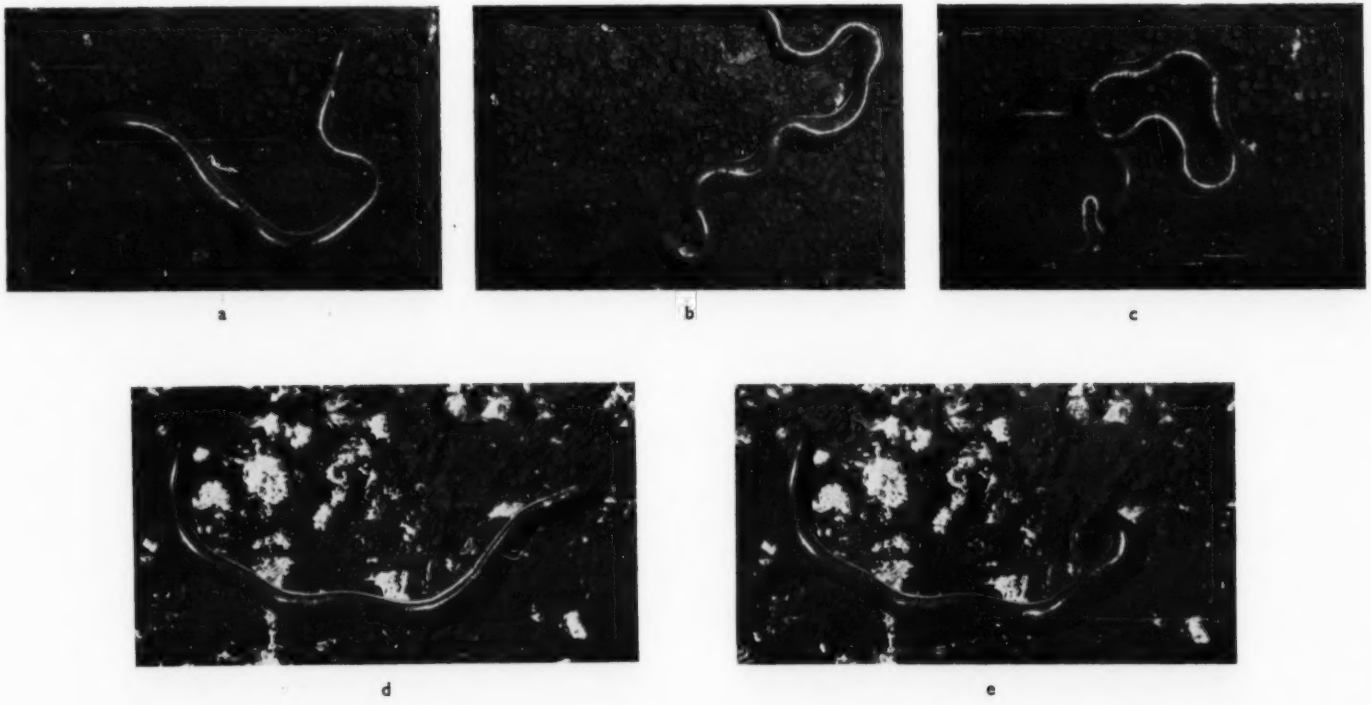
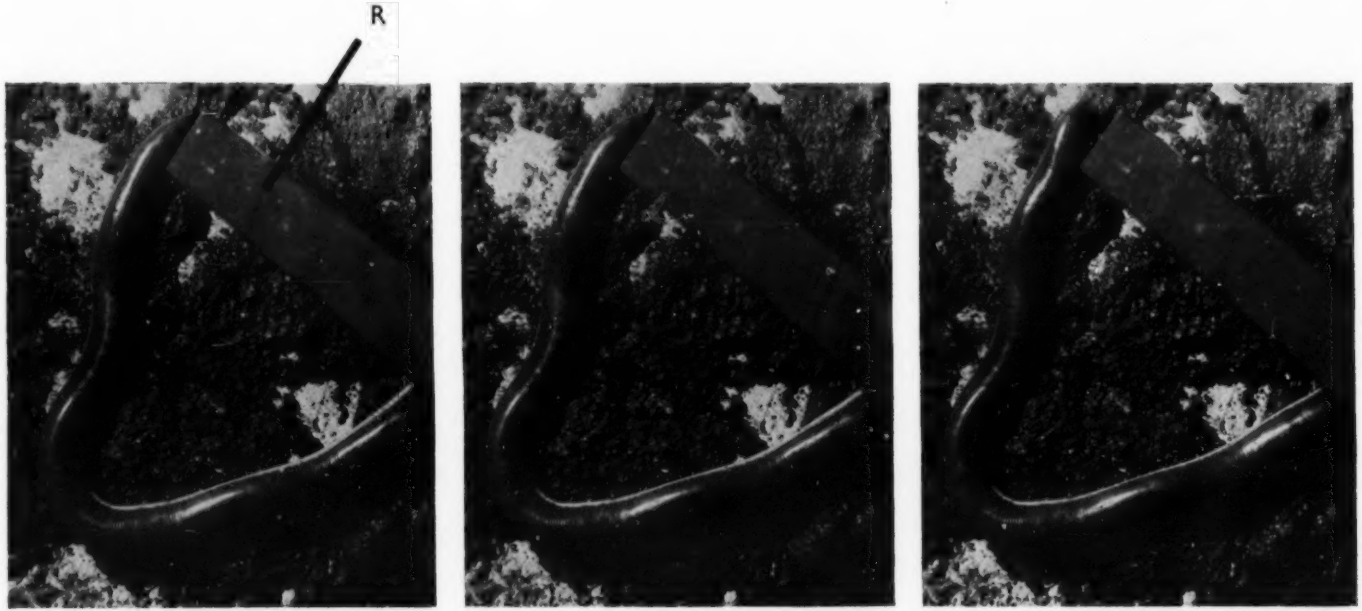


Ichthyophis glutinosus in observation box photographed with electronic flash (duration 1/800 sec.). (*a*) animal at rest in dark (almost never outstretched but usually bent as in picture); (*b*) animal roused by general illumination showing typical serpentine coiling preparatory to locomotion; (c) animal ingesting an earthworm and moving very actively; (*d*) and (e) animal with spinal cord cut about one-third of the distance from head to tail. (*d*) was taken about 20 sec. after the animal was exposed to general illumination and (e) about 35 sec. later; the animal is active in front of the cut but perfectly still behind.



Ichthyophis glutinosus photographed with electronic flash 3 days after transection of the spinal cord. The rod R with blackened sides transmits light from a concealed source 2 ft. away and illuminates an area 20 x 8 mm. The intensity of the flash is such that the illuminated area does not show up in the photographs. (*a*) Rod brought up to tail of resting animal in safe light before illumination; (*b*) 4 sec. after local illumination switched on; tail just beginning to move; (*c*) 10 sec. after local illumination began; tail has moved out of light.

THE SKIN AND NUCHAL EMINENCE OF THE WHITE RHINOCEROS

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(With 1 plate and 2 figures in the text)

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INTRODUCTION

Rhinoceros skin enjoys a unique and traditional reputation for density, inelasticity and impermeability—so much so that "the hide of a rhinoceros" is the conventional derogatory term applied in stigmatisation of the "thick skinned" individual who is impervious to blandishment and censure equally. Mere thickness of skin cannot explain the adoption of the rhinoceros as exemplar thereof, since other mammals (e.g. elephant, hippopotamus, giraffe) possess skins sufficiently thick. It is the extraordinary density, toughness and almost metallic inelasticity of rhinoceros skin which renders it unique among that of "pachydermatous" mammals.

On mere inspection, and in contrast to the soft, supple, hairy skin of the generality of mammals, rhinoceros skin (of any one species) appears remarkably "dead" or inactive, a dull, wrinkled, rough, dry and hairless armour-plating, more suggestive of oak-bark than of living animal tissue (Plate, fig. 1). Such first impressions are reinforced by the experience of field naturalists, hunters and menagerie keepers, which testifies to the difficulty of penetrating this formidable integument. Upon flaying, the detached hide is found to be tremendously thick, heavy beyond expectation and exceedingly difficult of manipulation. In the adult White or adult Indian Rhinoceros it may weigh a ton and require considerable manpower to drag it over even the smoothest ground. Its consistency is that of a sheet of thin steel (Heller, 1913) and it defies all attempts at folding. Its density and rigidity prevent its being worked, so that it remains without commercial value and (save for the manufacture from it of curios) of service to the taxidermist only.

It is these physical properties of resistance, density and inelasticity which have so impressed naturalists and, in popular opinion, have justified the choice of the rhinoceros as the acme of "thick-hidedness" or integumental imperviousness.

It is the principal purpose of this paper to describe the histological structure of White Rhinoceros skin and thereby to demonstrate the anatomical basis of its obtrusive physical properties. A second purpose is to establish clearly and finally the anatomical nature of the nuchal hump, a curious and distinctive external morphological character of this species.

This hump, *inter alia*, distinguishes the White Rhinoceros (*Rhinoceros simus* Burchell=*Ceratotherium simum* Gray) from the Black Rhinoceros (*Diceros bicornis* (L.)). It is a large, median nuchal prominence, present in both sexes, arising from the continuous surrounding skin upon a fairly well-defined quadrilateral base : it confers a distinctive convexity upon the cervical midline and extends, in the adult, from the occipital crest region almost to the withers, overlying therefore the spinous processes of the second to sixth cervical vertebrae. This notable external character of *Ceratotherium* received remarkably scant attention in the literature prior to Heller's (1913) classical monograph : if mentioned at all, its position was given imprecisely or its structure was left undefined.

Burchell himself, who first (1817) scientifically distinguished the White, or Square-lipped, Rhinoceros from its Black congener, omitted all reference to it, although his familiarity with it is apparent from his field drawings (see Cave, 1947).

Chapman (1868) figured the hump in a dead "Rhinoceros mohogu" and observed that "The back of the mohogu is tolerably straight, but the croop (?hench bone) is as high or higher than the withers ; has a huge square hump, a double navel, and the females a blind pouch near the vagina". Newton (1903) published photographs of dead specimens with the hump apparent, but gave no textual reference thereto. Trouessart (1909) published an unsatisfactory coloured drawing, showing a hump situated upon the withers, together with photographs of dead specimens wherein the hump (unmentioned in his text) is not distinguishable.

Gray (1867) listed among the external characters of *Ceratotherium* a "shoulder with a well-marked hunch", but was silent concerning the nature or extent of this "hunch".

Selous (1881) stressed the edibility of this part of the animal without detailing its position or nature. Bryden (1899), repeating Selous' account, stated that the hump was situated "just in front of the withers" : he said nothing regarding its constitution. Lydekker (1908) merely repeated earlier statements anent hump edibility. Somewhat surprisingly the hump is neither figured nor mentioned in accounts of the White Rhinoceros given by Cumming (1850), Buckley (1876), Drummond (1876), Sclater (1886, 1900), Bryden (1897), Thomas (1901), Beddard (1909) and Lydekker (1910) : the last author indeed gave a bad figure of the living animal devoid of all trace of hump. The classical monograph on mammalia by Flower & Lydekker (1891) merely quotes Selous regarding the succulence of the hump, nothing being said about

its nature or situation. It would seem that neither author was familiar with the living animal or with an accurately-mounted museum specimen. (In this connexion it may be noted that the first mounted example in the British Museum (Natural History) was set up in 1894, being one of two animals shot in Rhodesia in 1892 by R. T. Coryndon, the second specimen going to Tring Museum).

To the present time the nuchal hump of *Ceratotherium* has remained imperfectly known in zoological literature, its position being given either erroneously or but approximately, its constitution never, and its reputed succulence alone implying its non-skeletal nature.

As late as 1910 Roosevelt could comment that " it is a singular thing that scientific writers seem almost to have overlooked, and never lay any stress upon, the existence of this neck hump. It is on the neck, forward of the long dorsal vertebra, and is very conspicuous in the living animal ". Heller (1913) described this feature as " the prominent, rounded, fleshy hump upon the

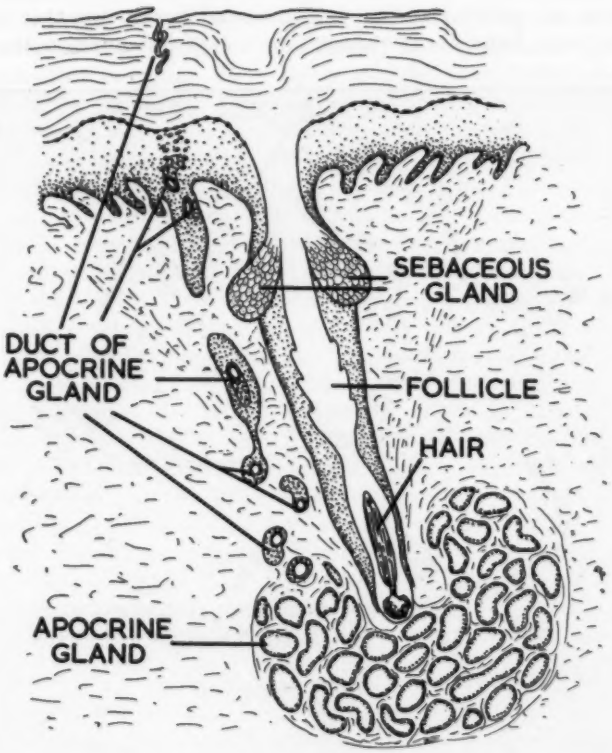

Fig. 1.—*Ceratotherium simum*. Composite histological drawing of epidermis and superficial part of dermis, to show (a) heavily keratinised, pigmented epidermis, (b) hair follicle with contained hair, (c) small paired sebaceous glands, (d) large apocrine sweat gland.

nape of the neck just forward of the withers ", stating that it is " purely a muscular structure and receives no support from the dorsal processes of the cervical vertebrae ". This is the first description (as apart from notice) of the precise location of the hump and a recognition of its non-skeletal or " muscular " nature.

That the hump is not muscular, but dermal, in constitution, was tentatively but correctly deduced by Bigalke *et alii* (1950) from observation of a young captive female animal. They recognised it to be " part of the skin " (because mobile upon palpation and neck movement) and concluded that, while its true nature " remains to be determined by anatomical dissection, there is little doubt that it is not entirely a muscular structure ". Herein the strictly dermal nature of the hump is established.

MATERIAL

The material studied and reported on here was obtained for us, under conditions of considerable difficulty, by Dr E. H. Williams, of Arua, West Nile District of Uganda, from an immature animal dispatched officially following its mutilation by native poachers. The nuchal hump, together with pieces of the skin, liver, kidney and stomach wall, was preserved in the field and

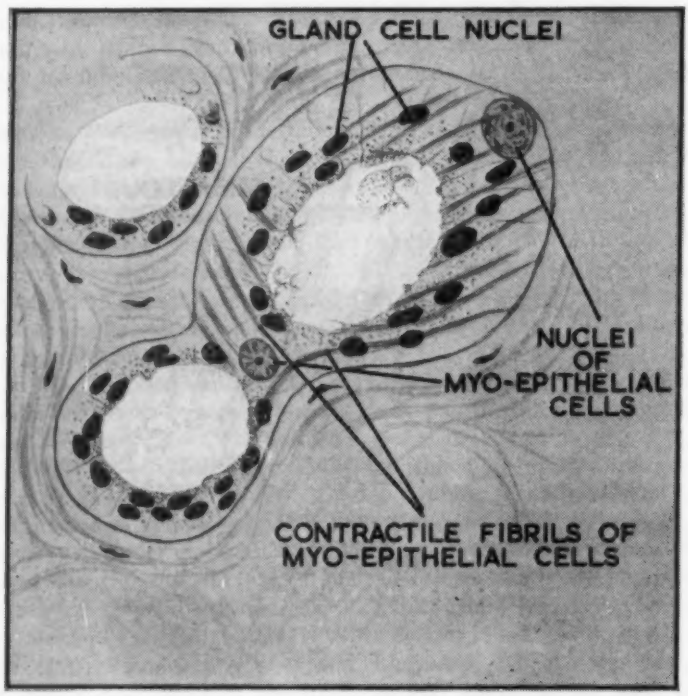

Fig. 2.—*Ceratotherium simum*. High power drawing of apocrine sweat gland to show myoepithelial cells.

reached the laboratory in good state. After due processing, the various tissues stained well (with haematoxylin-and-eosin, Mallory's triple stain, etc.). Macro-sections were made of the nuchal eminence (Plate, fig. 2) and micro-sections of the skin pieces. These last proved difficult to cut, and the best sections obtained varied in thickness from 15μ to 20μ. (As is invariable in this species, the stomach contained nothing save close-cropped grass. An inch-long filarial worm was deeply embedded in the conjunctiva).

The skin micro-sections, by reason of their relative thickness and, often, because of the obliquity of their plane of section, proved unsuitable for direct photographic reproduction. But careful study of successive sections permitted a reliable, if composite, picture of skin-structure (Figs. 1, 2) to be established.

THE SKIN OF *CERATOTHERIUM*

Micro-examination reveals the skin to be of typical mammalian constitution and to be anatomically organised so as to function as a responsive and protective organ subserving sensibility and excretion. It presents two striking specializations in the form of (a) a relatively thick and very dense dermis, and (b) the presence in the superficial layer thereof of large apocrine sweat glands. It is no inert outer wrapping, but a highly modified and physiologically active integument. The whole skin is attached to the deeper structures by non-resistant superficial fascia, which facilitates rapid and easy flaying. The superficial fascia is uniformly fat-laden, the thickness of the fat being as much as one inch on the dorsum of the trunk and as much as two inches on the ventral abdominal wall. So substantial a subcutaneous fat layer provides an ideal insulator for the conservation of body heat, besides topographically obviating that manifestation of costal processes and ridges seen in *Diceros*. (Chapman (1868) noted that *Ceratotherium* "becomes exceedingly fat by grass eating").

The epidermis is generally surprisingly thin (about 1 mm. in thickness) although in body regions not examined (feet, sacral eminence, etc.) it may be thicker : it is everywhere heavily cornified and sends down into the dermis hair-follicles, sebaceous glands and a specialised type of sweat gland. The stratum corneum equals or exceeds in thickness the stratum Malpighii : the cells of its deepest layer contain small discrete granules of melanin. A stratum lucidum is not everywhere apparent. The stratum granulosum averages some two cells in thickness. A section of the " thin " belly skin of our immature animal gave the following relative thickness of component layers :

epidermis = 1 mm.	stratum corneum	0·25 mm.
	„ lucidum	0·5 „
	„ granulosum + „ germinativum	0·25 „
dermis = 18 mm.		

The dermis generally is enormously thick (18–20 mm.) and is composed exclusively of densely felted collagen fibres, disposed at all angles (vertical, parallel and tangential) to the surface. The only elastic tissue within this layer is that present in the walls of the dermal arterioles. It may well prove to be

much thicker in parts of the skin not examined : on the nuchal eminence it attains (in this immature animal) a thickness of about 45 mm. Despite the extraordinary thickness of its dermal component, the skin of *Ceratotherium* enjoys a remarkable degree of vascularity : blood-vessels 1 mm. or so in diameter can be observed by naked eye to proceed from the superficial fascia and subcutaneous fat to enter recognizable channels (" dermal foramina ") in the deep aspect of the dermis, wherein they ramify to supply the remarkably large apocrine sweat glands and the bases of the hair follicles.

Hair follicles are present in all the skin micro-sections examined, including the skin of the nuchal eminence. Within the follicles are the stumps of hairs, in some instances confined to the depths of the follicles but in others extending up the follicle towards, or even almost to, the surface. Projecting hairs were not seen, but for a single tuft upon the summit of the nuchal eminence. Save for the eyelashes and the ear- and tail-fringes, hairs are not readily detectable on the body surface and are commonly said to be altogether wanting. Heller (1913) stated that the neonatal *Ceratotherium* is no more hairy than the adult, but Bigalke (1950) was able to detect body hairs in a young female up to the age of fifteen-and-a-half months. Grassé (1955), summarising the relevant literature on the Rhinocerotidae, refers merely to the hirsute form (*D. lasiotis*) of *Didermocerus*, to the ear- and tail-fringes of extant species and to the presence of hairs " dans les plis de peau qui séparent les grandes plaques cuirassées quand celles-ci existent ".

The standard authoritative zoological treatises provide no authentic information nor any histological evidence touching the presence of body-hairs in the Rhinocerotidae. Neuville (1927), in a special study of rhinoceros skin, regarded it as hairless, but stated that hairs may be detected around the horn base in the three Asiatic species : he made no comparable reference to the two African species and he nowhere adduced any histological evidence in support of his general thesis that excessive keratinization (a reaction to external environmental stimulation) was the factor responsible for " depriving " rhinoceros skin of the normal mammalian equipment of glands and hairs.

Present findings suggest that an original sparse external hairy endowment becomes reduced with age, either from the mechanical abrasion of the projecting portions of the body hairs or from natural atrophy consequent upon the development of a substantial layer of subcutaneous fat.

The base of each hair follicle is embedded in the superficial part of the thickened, collagenous dermis and is surrounded by an impressively rich network of sweat gland tubules. Nearer the epidermis, each follicle is provided with a pair of small, piriform, rather feebly developed sebaceous glands (Fig. 1) which appear less numerous in the sections than do the sweat glands.

The ordinary, small type of sweat gland is nowhere apparent in the skin micro-sections studied. Instead, relatively enormous apocrine sweat glands constitute a striking histological feature of the skin. Not over-abundant, these epidermally-developed glands are of uniformly large size and are disposed in open basket fashion around the bases of the hair follicles. The gland cells are arranged in a single layer ; their oval nuclei stain with moderate intensity by haematoxylin and contain coarse granules of chromatin. The sweat ducts

spiral surfacewards, being fairly capacious in their intradermal, but narrower in their intra-epidermal, extent. Both the glands and their ducts are characterised by enormous, ectodermally-developed myoepithelial cells, which provide one of the most striking anatomical characters of the skin. These lie (a) between the gland epithelium and the basement membrane, (b) in helicoidal disposition around the ducts. They furnish the mechanism whereby the large apocrine sweat glands may effect rapid physiological response to sudden functional demands.

The glands are highly vascular, being supplied by relatively large arterioles, which tunnel the dense dermis and branch freely therewithin to reach individual glands.

Thus, on direct histological evidence, the skin of *Ceratotherium* is very much " alive " and singularly well equipped to discharge the several functions of typical mammalian skin. The delicate, pigmented epidermis serves as an external sensorium ; the enormously thickened dermis is mechanically protective ; the substantial layer of subcutaneous fat prevents loss of body-heat, and the remarkably large and well-vascularised apocrine sweat glands provide for the rapid and copious discharge of body-fluid.

In the life of so large an animal, efficient sweating must obviously play an important role, for only by profuse and rapid sweating can any sudden, deleterious rise in body temperature be prevented. Recorded information concerning the sweating capacity of *Ceratotherium* is wanting. Normally the animal seeks cover during bright sunshine and displays its maximal activity in dull daylight or in cool, cloudy or humid weather. When persistently disturbed in hot sunshine it can be observed to sweat freely, as one of us (D.B.A.) has noted.

Its capacity, upon violent exercise, for releasing a sudden and copious discharge of sweat is authenticated by Coryndon, who described (in Bryden, 1899) how a young specimen, roped and struggling on the ground, " began to sweat freely, the moisture dripping off it as though a bucket of water had been thrown over it ".

The machinery for such sudden and copious discharge of body-fluid lies obviously in the huge apocrine sweat glands and their enormous, attendant myoepithelial cells, which constitute a structural adaptation admirably designed to subserve the functional requirements of this animal.

(The total daily volume of body-fluid discharged, either in the form of sweat or of urine, is, of course, unknown. But in this connexion the peculiar mode of micturition in *Ceratotherium* is noteworthy. The animal does not stand and stale by a total contraction of the bladder : instead it effects a discontinuous series of partial badder contractions, spraying small squirts or " puffs " of urine, at irregular intervals during its progress through the bush or around its feeding ground. The animal is prone to return along the trail so " blazed " by means of this discontinuous urine-spraying, for olfaction is its most exquisitely developed and dominant special sense and its principal means of identifying and locating external objects).

THE HUMP (NUCHAL EMINENCE)

The nuchal eminence of our immature specimen (Plate, fig. 2) is revealed by dissection to be a non-fatty, non-muscular, purely cutaneous structure, a dermal node or plaque resulting essentially from an extraordinary, localised and very considerable augmentation of the nuchal dermis. Over the eminence the epidermis is a little thickened. In the middle of the eminence the collagenous dermis virtually doubles its thickness elsewhere and attains an average of 42–44 mm. (In the adult animal the dermal thickness here must be relatively much greater, in proportion to the greater bulk of the eminence). It is this thickened dermis which the older travellers found so palatable. On the summit of the eminence a tuft of hairs is present. The skin of the eminence resembles the skin examined from other parts in its like manifestation of hair follicles, small sebaceous glands and large apocrine sweat glands. No obvious explanation is readily forthcoming concerning the significance of this nuchal dermal node or eminence, so curiously confined to this species of rhinoceros. It is not present at birth but develops shortly afterwards, according to Bigalke (1950), from the gradual coalescence of three cervical midline cutaneous thickenings. These nodules or plaques begin to appear from two to four weeks after birth and their confluence is completed by the sixth month.

SUMMARY

1. The skin histology of an immature White Rhinoceros is described. The thin epidermis has the customary layers, the deepest layer being pigmented. It sends into the dermis hair follicles (containing hair shafts), small sebaceous glands and large apocrine sweat glands characterised by enormous ectodermally-developed myoepithelial cells.

2. The exceedingly thick and well vascularised dermis is exclusively collagenous in composition.

3. The hump or nuchal eminence is merely a local augmentation of the dermis (nuchal node).

ACKNOWLEDGMENTS

Our gratitude is extended to Dr E. H. Williams for his collection, under great difficulties, of the material forming the basis of this study, to Dr John Aumonier for his generous supervision of histological findings, and to the Librarian of the Zoological Society of London and his Staff.

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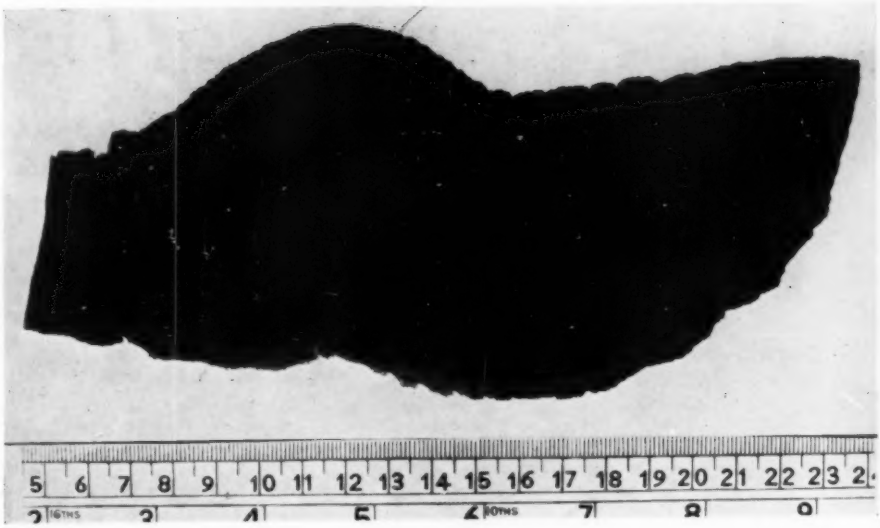

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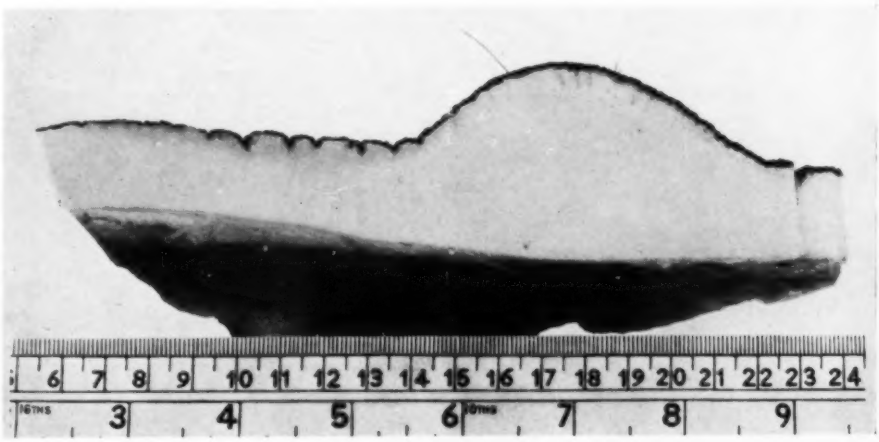

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EXPLANATION OF PLATE

Fig. 1.—*Ceratotherium simum.* Nuchal eminence (hump) of immature animal, superficial aspect in lateral view to show appearance of the skin. A hair is visible on the summit of the eminence. Cm. scale.

„ 2.—*Ceratotherium simum.* Nuchal eminence of same animal, longitudinally sectioned to show (a) projecting summit hairs, (b) the relatively thin epidermis, (c) the great depth of the dermis. Cm. scale.

REVISION OF THE CLARIID CATFISHES OF NYASALAND, WITH A DESCRIPTION OF A NEW GENUS AND SEVEN NEW SPECIES

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[Accepted 10th June 1958]

(With 7 figures in the text)

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INTRODUCTION

With regard to the fish fauna, the country of Nyasaland forms a compact zoogeographical entity. Eastwards the political boundaries are in Lake Nyasa, which forms so great an area of the country, and to the west along the divide between the Loangwa River and the streams flowing into the Lake. Massive ranges to the north ensure that all streams flow lakewards, while the Shire River, draining the Lake into the Zambesi, is divided by the Murchison Cataracts, which form an effective barrier for most Lower Zambesi fishes. Thus much the greater part of the country lies within the Nyasa drainage basin, only a small portion to the south-east draining towards the east.

Clariid catfishes are abundant in Nyasaland, forming an important food for the local peoples. During the course of a fisheries survey of the northern part of Lake Nyasa undertaken by the Joint Fisheries Research Organisation in 1954 and 1955 it became clear that the species endemic to the lake were more truly lacustrine and colonised the lake more fully than did those with a wider distribution. Two of the endemic lacustrine species were described by Worthington (1933) and a third by Jackson (1955), who pointed out that the species endemic to the lake formed a species-flock of closely related species. Such species-flocks of fishes are known from several of the great lakes of Africa ; they are characterized by close relationship, very similar morphological structure and, in general, strict adherence each to a narrow habitat.

In Lake Nyasa species-flocks were previously known from the cichlid genera only, such as *Haplochromis* and *Tilapia*. Lowe (1953) has shown how slight morphological differences, and how important environmental considerations, are for the endemic species in the latter genus. During the survey of

northern lake fisheries fishes of the genus *Clarias*, which were being caught in numbers in gill-nets set in deeper waters than had previously been the practice, proved to be most puzzling ; it soon became clear that the opinion given by Lowe (1952) that many of the *Clarias* species were as yet undescribed was perfectly correct. A study of this genus was therefore commenced, although it was not possible, due to fishery survey commitments, to spend as much time on it as the author would have liked, and problems of preserving adequately specimens of these usually large animals in the field in a humid tropical climate proved to be most difficult. Further work is needed on these difficult but interesting fishes.

NOTES ON MEASUREMENTS

Standard length is from the tip of the snout to the furthest end of the caudal peduncle. *Length of head* is from the tip of the snout to the end of the occipital process. *Snout* is measured in the sagittal plane, from the anterior edge of the eye to the level of the tip of the snout. *Gill-raker* and *gill-filament lengths* are those of the longest raker or filament. *Gill-raker counts* are those of the total number on the outer branchial arch. *Tooth-bands* are measured for half the total length, from the outer edge to the beginning of the gap, if any, otherwise to the central symphysis. *Widths of tooth-bands* and of *caudal peduncle* are the greatest widths. *Length of caudal peduncle* is measured from the end to a line down at right angles from the posterior base of the dorsal fin. *Width of mouth* is measured from the end of the labial groove in the maxillary barbel on either side. Where a pair of barbels are of different lengths the longest is measured.

All holotypes, paratypes and other specimens mentioned of new species have been deposited in the British Museum (Natural History).

THE FAMILY IN NYASALAND

Genus *Clarias* [Gronovius 1763, Zoophyl. I, 100]. Scopoli 1777, 455.

Excluding that part of the Shire River lying below the Murchison Cataracts, which has a Lower Zambesi fauna, there are so far discovered in Nyasaland four members of the genus, which all have a wider distribution. These are *Clarias mossambicus* Peters, *C. mellandi* Boulenger, *C. theodorae* M. Weber and *C. carsonii* Boulenger. The latter are the only two known in the country which have the pelvic fins nearer the tip of the snout than the end of the caudal peduncle. None have colonised Lake Nyasa to anything like the same extent as the endemics.

CLARIAS CARSONII Boulenger

Boulenger (1903) p. 362 ; (1907) p. 1077 ; (1911) p. 243, fig. 202. Jackson (1955) p. 683.

Two specimens in the British Museum presented in 1905 by Dr W. A. Cunnington with locality as from " Lake Nyasa ", since unrecorded from the country until the J.F.R.O. survey.

A lithophilic species, found in Nyasaland to be plentiful in high mountain streams and torrents, on the bottom among stones, living on insects and animal debris swept down with the current. It has not been found in Nyasa-

land in any situation lower than about 3000 feet above sea level, nor in water of a higher temperature than 18°C. It seems certainly to be the case, therefore, that Dr Cunnington's specimens are not from Lake Nyasa as recorded but from some stream in the north Nyasa highlands, a locality which, because of the precipitous nature of the country, may actually be quite close to the lake.

Clarias carsonii from Nyasaland is a small catfish, very active in escape when pursued, black in colour with a very short head and markedly cylindrical body. Its preference for a high-altitude fluviatile habit is very marked here, though elsewhere in Africa this does not always appear to be the case. The family in this continent in general, and these small stream-dwelling species with anteriorly placed pelvic fins in particular, are in need of another revision, when no doubt it will be found that fewer species and more subspecies are involved.

CLARIAS THEODORAE M. Weber

Weber, M. (1897) p. 150. Boulenger (1907) p. 1089 ; (1911) p. 262, fig. 218. Worthington (1933) p. 311 (synonymy outside Nyasaland). Lowe (1952) p. 87 (record from Lake Chilwa).

C. theodorae is a fish of lower reaches of rivers and of calm shallow lagoons, found in Lake Nyasa only where similar conditions prevail. In situations such as the Kampambe lagoon near Kota-Kota and the Limpasa Dambo (marsh) near Nkata Bay it is an abundant little fish, quite important in the economy of lagoons and marshes. It feeds on insects and other small animals and seems always to be associated with stagnant or slow-flowing water and aquatic vegetation, among which it coils up and hides.

Native names : " Niumbe ", " Kobo ".

CLARIAS MOSSAMBICUS Peters

Peters (1852) p. 682. Boulenger (1907) p. 1071 ; (1911) p. 232, fig. 195. Worthington (1933) p. 307 (status of *C. gariepinus* (Burch.)). Bertram *et al.* (1942) p. 48. Lowe (1952) p. 71. Jackson (1955) p. 683.

This is essentially a fluviatile species ; it is common in the middle and lower reaches of all Nyasaland rivers, especially where these are stagnant or slow-flowing, and has colonised the lakes, Lake Nyasa in particular, only where conditions are similar. It is much the commonest clariid in Lakes Chilwa and Malombe, and is the only one so far recorded from Lake Kazuni, where it is extremely abundant. In Lake Nyasa it is quite common in the south-east arm, but is not otherwise to be found in the lake except where similar sheltered and shallow conditions prevail, such as at Bana, or in river mouths, lagoons and shallow bays. It is not therefore true to say that it is the commonest clariid in Lake Nyasa ; it has probably often in the past been confused with granular-headed endemic species (see especially *Bathyclarias nyasensis* (Worthington) below).

Lowe (1952) was undoubtedly correct in calling *C. mossambicus* an " omnivorous scavenger ", although the bias is towards flesh where possible. They are often taken on lines. In August 1954 Mr Harding of the survey staff noticed a number floundering round the decaying carcase of a crocodile lying on the shore, in a few inches of water, at the mouth of the lagoon at Bana. So anxious were the fish to get at the flesh that they were coming right up to

the shore line with most of their bodies exposed. A set line baited with crocodile flesh caught thirteen fish weighting 70¼ lb. after three hours in the water, while a hand line caught six fish weighing 30 lb. in twenty minutes.

Native name : " Mlamba ".

CLARIAS MELLANDI Boulenger

Boulenger (1905) p. 644 ; (1907) p. 1074 ; (1911) p. 238, fig. 198. Worthington (1933) p. 310. Bertram *et al.* (1942) pp. 49, 117.

This species was first recorded from anywhere in the Zambesi system by Worthington from eight specimens collected by Dr Cuthbert Christy from Fort Maguire to the Shire outflow, Lake Nyasa. Bertram *et al.* record six specimens from Fort Johnston and the southern lake. It was collected from more northern points at the edge of or near Lake Nyasa by the J.F.R.O. survey, from the mouths of the Ruhuhu River on the north-east coast and the Luweya on the north-west, and one specimen from the Banga River about ten miles inland of Nkata Bay. It does not appear to be at all abundant, but no doubt occurs wherever habitat is suitable. As recorded elsewhere, the chief article of diet appears to be molluscs.

Native name : " Mlamba ".

Genus **BATHYCLARIAS** gen. nov.

Dorsal and anal fins composed of soft rays, with no dorsal adipose fin present. Pelvic fins approximately midway between tip of snout and root of caudal. Sides of head enclosed in bony shields. Eyes lateral, forming part of the outline of the head as seen from above. Fan-shaped structures on posterior end of gill-filament series well developed on each gill arch but arborescent suprabranchial accessory breathing organ much reduced to a stump on fourth arch, bearing six or fewer short projections. Suprabranchial chamber almost empty, its vascular epithelium very much reduced or absent.

Type species : *Clarias longibarbis* Worthington (1933).

Distribution : Lake Nyasa, Africa.

This new genus comprises the three previously described species mentioned in the introduction and seven new species which are described below. These ten species form a species-flock endemic to Lake Nyasa where they occupy a variety of habitats down to the limits of dissolved oxygen, and, so far as is known, never venture out of the lake into the adjacent rivers and swamps. While granular-headed specimens are superficially very similar to *Clarias mossambicus* and *C. mellandi*, occasionally found in less open-water parts of the lake, they may readily be distinguished from them by the lateral position of the eye in the case of *Bathyclarias* , when one of these is viewed from above the eyeball forms a bulge in the silhouette of the head, whilst in other clariids the eye is situated dorsally rather than laterally, and forms no part of the outline of the fish as seen from the dorsal aspect.

The lateral eye position and the reduction of the dendritic accessory breathing organ, so that the suprabranchial chamber is virtually empty, though the " gill-fans " are well developed, are extremely interesting features. They are undoubtedly connected with the open-water lacustrine habit adopted

by the genus as a whole, but the problems of anatomy, speciation and evolution involved lie outside the scope of the present paper. The accessory air-breathing structure of these fishes are being further studied by Mr P. H. Greenwood of the British Museum (Natural History).

TABLE 1

Number of gill-rakers compared with raker/filament ratios in *Bathyclarias nyasensis* and *B. worthingtoni* of varying lengths.

Bathyclarias nyasensis					*Bathyclarias worthingtoni*				
T. Length (mm)	St. Length (mm)	Gill-rakers on first arch (total)	Raker / Filament	Length (mm)	T. Length (mm)	St. Length (mm)	Gill-rakers on first arch (total)	Raker / Filament	Length (mm)
280	—	135	7/9 = 0·78		80	72	29	1·5/2·5 = 0·60	
334	—	157	9/11 = 0·82		256	231	70	3·5/7·0 = 0·50	
400	—	188	10/13 = 0·77		264	236	75	3·5/7·5 = 0·47	
596	544	220	19/20 = 0·95		535	473	100	8/16 = 0·50	
605	552	237	18/20 = 0·90		—	540	107	12/19 = 0·63	
652	585	214	21/25 = 0·84		645	564	111	15/29 = 0·52	
659	592	216	18/20 = 0·90		—	611	130	10/32 = 0·31	
673	597	221	21/23 = 0·91		685	627	130	13/21 = 0·62	
—	638	236	26/28 = 0·93		771	683	110	17/25 = 0·68	
725	641	233	25/27 = 0·93		814	705	117	13/34 = 0·38	
—	700	246	31/34 = 0·91						
800	—	220	34/33 = 1·03						

BATHYCLARIAS NYASENSIS (Worthington)

? Keilhack (1908) p. 169 (*Clarias capensis* forma *mossambicus*). Worthington (1933) p. 308, fig. 9 (*Clarias nyasensis*). Jackson (1955) p. 683.

The first described of the species endemic to Lake Nyasa, this is also the most abundant and most economically important clariid in the lake. It is a very variable species, particularly in the degree of granulation on top of the head. Specimens occur which are practically smooth-headed ; these also have the top of the head more fleshy, the " squamosal " bones more humped, and the sides of the head relatively wider at the operculum than is the case of the typical granular-headed forms, which have the sides of the head roughly

parallel and the top roughly cylindrical without humping. Such smooth-headed fishes would be considered distinct did they not appear to be connected by numerous intermediate stages with the granulate form. The colour is brown or red-brown, sometimes purplish-brown especially in the smooth-headed facies with the under-surface white or cream-white, sharply demarcated from the upper. The dorsal and paired fins usually have light cream margins tinged with red, and there is a distinctive cream crescent on the base of the caudal fin, parallel to its margin.

I have examined the type and other specimens in the British Museum, which are typically granular-headed, and have deposited there one entire specimen and a head which show the smooth-headed features referred to above. No ecological differences could be found. Critical examination of a long series may reveal specific differences, but it is not proposed here to do more than to record the observed variations within Worthington's species.

As Worthington (*loc. cit.*) has remarked, the thirteen specimens of *Clarias* examined by Keilhack which are from north lake Nyasa and which he named *C. capensis* forma *mossambicus* may, at least in part, be referable to this species. If possible this material should be re-examined, having regard to such features as the position of the eye and the length and number of the gill-rakers.

B. nyasensis has very long and close-set gill-rakers, which vary in number with the growth of the fish (Table 1), and feeds largely (but not entirely) on plankton and also on the emergent stages of the lake-fly *Corethra edulis* Milne-Edwards when these hatch out in swarms over the northern lake. Other insects and fish are sometimes taken, though it is not a consistent flesh-eater. It is almost invariably taken in gill-nets, usually in deep water down to as much as 70 metres and practically never on lines. It forms a large part of gill-net catches in the northern lake but is also found in the south-east arm, so is a wide-spread species within the lake.

Native name "Sapuwa".

BATHYCLARIAS LOWEAE sp. n. (Fig. 1)

Depth of body 5·5 to 6·5 in total length ; length of head 3·2 to 3·6 times in standard length ; width of head 4·6 to 5·3 in standard length, its upper surface smooth or moderately granulate ; occipital process blunt. Frontal fontanelle narrow, partly grown together, 6 to 7 in length of head ; occipital fontanelle small, oval, 29 to 32 in length of head. Eye lateral, 17 times in head length, 10 times in interorbital width. Width of mouth equal to or slightly less than interorbital width. Length of snout 3·7 (in type specimen) to 4·4 in length of head. Length of nasal barbels 2·2 to 4·2, of maxillary barbels 1·3 to 1·6, of inner mandibular 2·8 to 3·7, and of outer mandibular 1·7 to 2·2 times in length of head (figures for type given last). Half praemaxillary tooth-band 4·4 to 4·5 times in head length, its width 8 to 11 in half length ; vomerine tooth-band width 8 to 9 times in half length ; a gap present ; teeth pointed. Gill-rakers very long and close-set, 236 to 253 on anterior arch, about as long as gill filaments. Dorsal 63 to 65, its distance from the occipital process 3·7 to four times in head length ; anal 49 to 53, distinctly separate from caudal. Caudal fin very large, floppy, its width

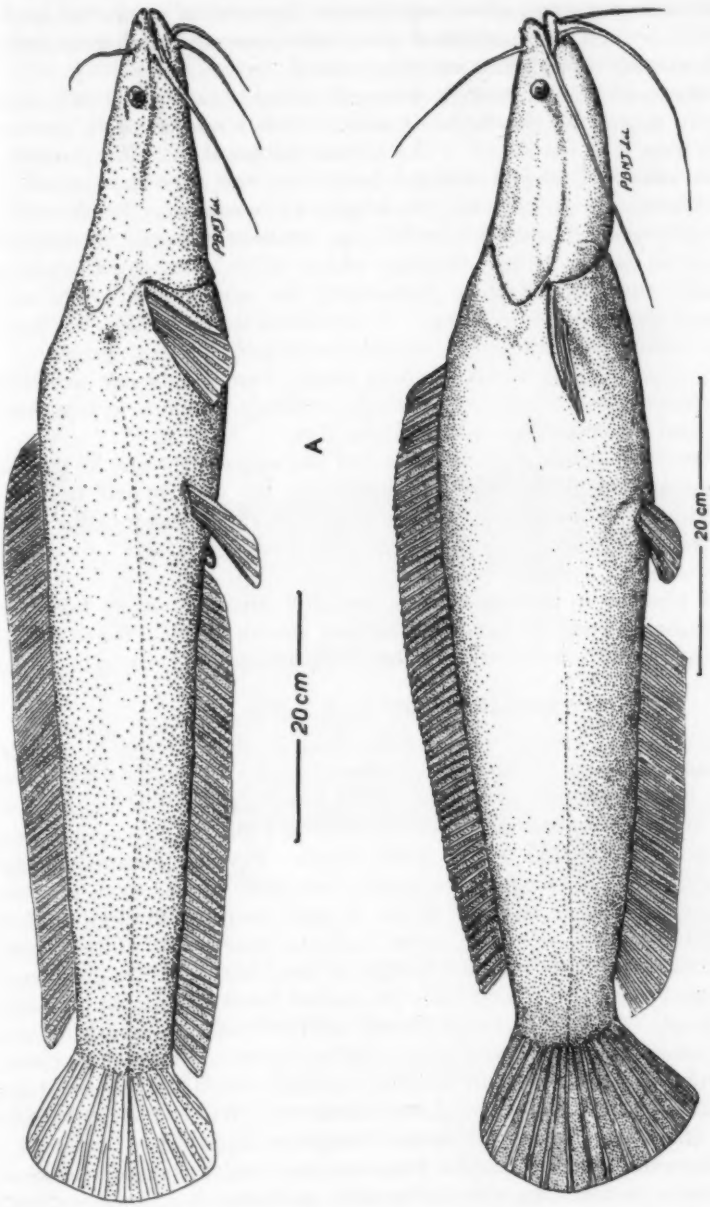

Fig. 1.—*Bathyclarias loweae* gen., sp. n. A. Type, male. B. Another specimen, female.

1·4 to 1·7 in length of head. Caudal peduncle flat and markedly deeper than long, its length 0·7 to 0·9 times its width. Pectoral fin large, with a strong spine, about equidistant from end of snout and root of caudal. Colour dark brownish-black or reddish-puce with darker marblings, with no lightening of colour on ventral surface.

Body deep and heavy, strongly humped on either side of anterior dorsal, falling away sharply to the flattened head. Closely related to *B. nyasensis*, but differs from it in the length of the barbels, longer than in *B. nyasensis* of comparable size, the deeper, humped body, and the wide flat caudal and caudal peduncle. In *B. nyasensis* the length of the caudal peduncle is about 1·2 times its depth. In addition to the type, another damaged specimen has been deposited in the British Museum which differs from the type in the shorter snout and longer barbels, particularly the nasal barbel. The colour is lighter and the head not granulate. It is referred to this species, but further work may warrant separating the two specimens into different species.

The holotype a male, 940 mm. in total length, from deep water near Nkata Bay. Another specimen, differing as above, a female, 775 mm. in total length (Fig. 1, A and B), from deep water, Nkata Bay.

Not abundant, taken in deep water but also apparently one of the more pelagic clariids, seen far out and recognisable by the massive tail, feeding on emergent swarms of the lake fly, *Corethra edulis*. The stomach of the type is crammed with this food.

Native name : " Nkomo ".

I have pleasure in naming this species after Miss Rosemary Lowe, now Mrs R. McConnell, who in her single-handed survey of the *Tilapia* of Lake Nyasa drew attention to the large number of clariid species existing in the lake.

BATHYCLARIAS ILESI sp. n. (Fig. 2)

Depth of body 9·0 in total length ; length of head 3·3 in standard length ; width of head 4·7 times in standard length. Head 1·4 times as long as broad ; its upper surface granulate and not fleshy ; squamosal bones scarcely humped ; occipital process angular. Interorbital width 1·7 in length of head ; distance between nostrils 3·7 in head length. Frontal fontanelle dagger-shaped, 4·6 in head length ; occipital fontanelle small, oval, 22 times in head length. Eye lateral, 16 times in head length, 10 times in interorbital width. Width of mouth about equal to interorbital width. Length of snout 4·1 times in head length. Length of nasal barbels 4·1 in head length, of maxillary 1·8, of outer mandibular 2·4, and of inner mandibular 3·7 times in length of head. Half praemaxillary tooth band 4·9 times in length of head ; width 5·2 times in length of half band ; half vomerine tooth-band 4·5 times in head length ; width 6·0 in half length ; vomerine teeth with a small gap ; teeth pointed. Gill rakers long and fairly close set, 170 to 184 in total number, length of gill rakers about 0·8 times in length of filaments. Dorsal 67, its distance from occipital process 3·8 times in head length ; anal 52, distinctly separate from caudal. Length of caudal peduncle 1·1 times its width. Colour jet black, slightly lighter black-green marbling beneath. Gill filaments purplish-black. A black removable mucus over body.

Very close to *B. nyasensis*, from which it is separated by the relatively fewer gill-rakers (*B. nyasensis* of the holoptype's size has at least 220 gill-rakers), relatively shorter gill-rakers, black coloration and removable black mucus, dark gill-filaments, wider tooth-bands.

One specimen, holotype, female with ripe gonads and 730 mm. long, from Nkata Bay between 20 and 50 metres depth. The stomach was empty. Another, male, paratype, 650 mm. long.

Native name : " Black Sapuwa ".

I have pleasure in naming this new species after Mr T. D. Iles, a colleague on the survey, who took a keen interest in the *Clarias* systematics, drew my attention to the existence of the " Black Sapuwa " and collected these specimens for me.

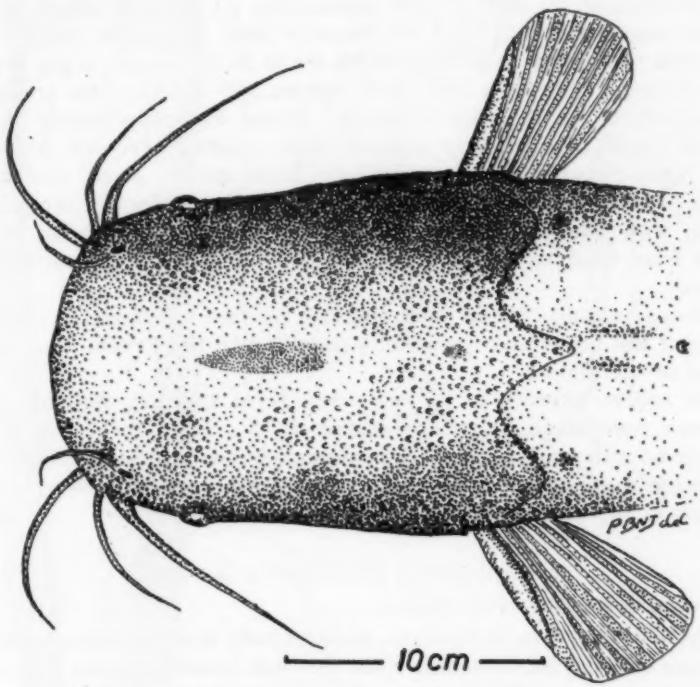

Fig. 2.—*Bathyclarias ilesi*, gen., sp. n. Type, female.

BATHYCLARIAS LONGIBARBIS (Worthington)

Worthington (1933) p. 309 (*Clarias longibarbis*).

The first described of the short gill-rakered group of Nyasa endemics, this species seems to live a more active and mid-water existence than do most species in the genus. It is a fish eater and is often taken on lines. It is a species variable in the shape of the head and height of the dorsal fin, and it is possible that further work will reveal the presence of two closely related species.

The type in the British Museum is an exceptionally large specimen, most individuals being considerably smaller.

Native name : " Kabwili ".

BATHYCLARIAS ROTUNDIFRONS sp. nov. (Fig. 3)

Depth of body five times in total length ; length of head 3·8 in standard length ; width of head 5·0 in standard length. Head 1·3 times as long as broad, its upper surface very smooth and fleshy ; occipital process blunt. Frontal fontanelle narrow, obscured by the fleshy skin ; occipital fontanelle small, obscured. Eye lateral, fairly large, 12·3 in head length, 7·5 in interorbital width. Width of mouth less than interorbital width. Length of snout 5·1 in length of head. Length of nasal barbals 4·3, of maxillary 1·7, of outer mandibular 2·1 and of inner mandibular 3·1 times in length of head. Half praemaxillary tooth-band 4·9 times in head length, its width 7 in its half-length ; vomerine tooth-band width 5·8 in its half-length, a gap present ; teeth pointed. Gill-rakers very short, sparse, 105–115 on outer arch, about one-third of the length of the filaments. Dorsal 64, spines heavily obscured by flesh ; anal 53, distinctly separate from caudal. Pectoral fin with a strong spine ; pelvic about equidistant from end of snout and root of caudal. Caudal peduncle about as long as broad. Headed rounded and chubby, with smooth curving contours ; body almost circular in cross section from head to middle of dorsal. Colour grey thickly marbled with dark grey.

Holotype a male, 681 in total length. Another specimen, 510 mm. long with head less fleshy and rounded, is deposited in the British Museum, and referred to this species.

This species appears to be rare, only three typically rounded headed specimens were taken, at over 40 metres depth where the bottom is rocky interspersed with sand. A few fish bones found in one stomach, others empty. It differs from all others in the fleshy rounded shape, chubbiness of the head, shortness of the snout and the relatively large size of the eye.

BATHYCLARIAS FOVEOLATUS (Jackson)

Jackson (1955) p. 681 (*Clarias foveolatus*).

Unique in the genus in its squat, humped body with the skin covered with numerous circular flat-bottomed pits, this well-marked species has a deep-water habitat, from 30 metres to all depths at which dissolved oxygen is present. It appears to have a marked preference for rocky bottoms, and has been found at Nkata Bay, Likoma Island, Bandawe and other northern lake localities with deep water and a rocky bottom. Living specimens caught in the deep-set gill nets were found on land to have the curious habit of raising the forepart of the body clear of the ground on the pectoral fins. This was not observed in other clariids, and may be connected with a predatory habit, the fish lurking among rocks and launching itself suddenly on its prey. The diet seemed to consist solely of fish.

Fairly often caught in deep-set gill nets and well known to local people,

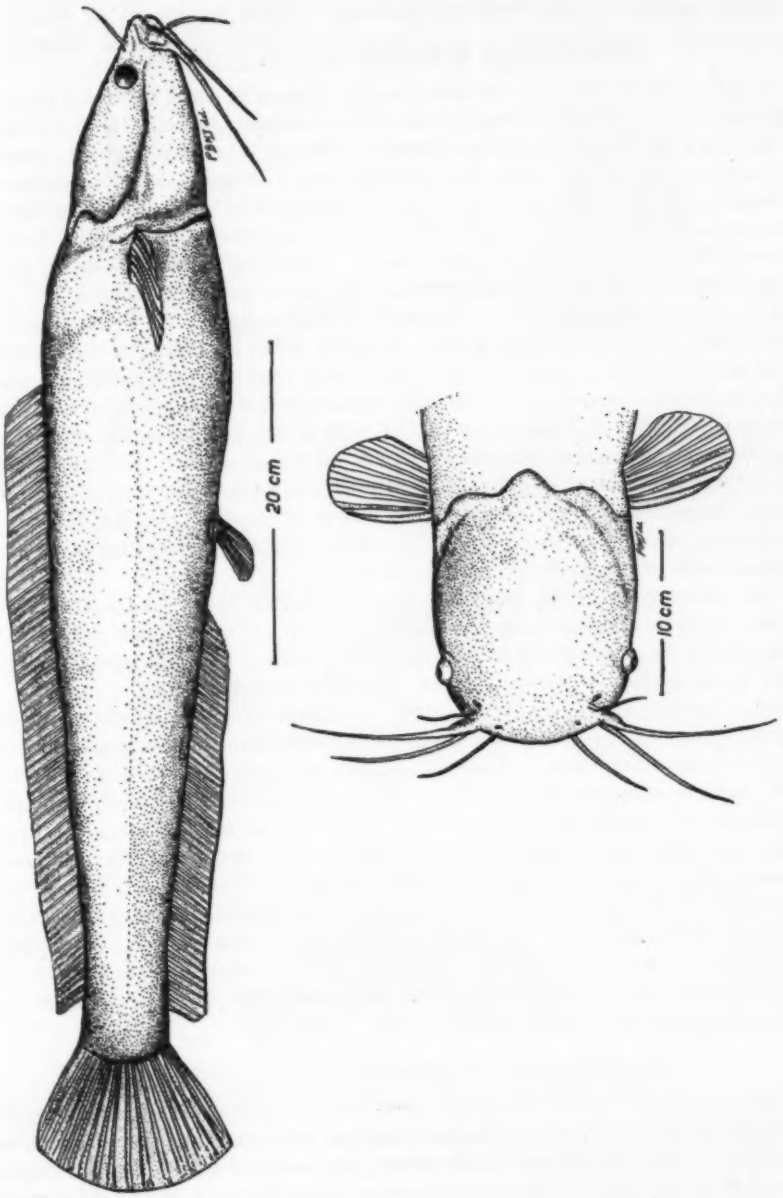

Fig. 3.—*Bathyclarias rotundifrons* gen., sp. n. Type, male.

who do not, however, eat it, as they connect the pitted skin with the pock-marks caused by small-pox in humans.

Native name : " Chimwamapumba ".

BATHYCLARIAS EURYODON sp. nov. (Fig. 4)

Depth of body 7·4 to 8·4 in total length ; length of head 3·2 to 3·3 times in standard length ; width of head 4·9 to 5·0 in standard length. Head 1·5 times as long as wide, its upper surface smooth. Occipital process angular ; frontal fontanelle a narrow slit 5·8 to 6·0 times in length of head, occipital fontanelle reduced or absent. Eye lateral, 15·7 to 17·0 in length of head. Width of mouth about equal to interorbital width which is 1·8 to 1·9 times in head length. Length of snout 3·6 to 3·8 times in length of head. Length of nasal barbels 2·0 to 2·3 times in head length, of maxillary 0·9 to 1·1 times, of outer mandibular 1·4 to 1·6 and of inner mandibular 2·2 to 2·4 times in head length. Half praemaxillary tooth-band 5·2 to 5·3 in head length, its width 4·3 in head length, 1·6 to 2·2 in its own half-length. Vomerine teeth in a very broad curved patch, the teeth granular and so close as to be virtually fused together. Mandibular teeth also in a broad band. Gill-rakers 94 to 112, very short, only about one-third the length of the filaments. Dorsal 60 to 69, its distance from occipital process 4·2 to 4·9 times in head length ; anal 49 to 54, distinctly separated from caudal. Caudal longer than deep, its length 1·1 times its depth. Pectoral fin with a moderately strong spine ; pelvic fin about midway along body. Colour blackish with grey marblings, lighter beneath.

The holotype a male, 545 mm. in total length, from Nkata Bay, Lake Nyasa, with inactive gonads. A single paratype another male, 505 mm. in total length, from Nkata Bay, with inactive gonads. Another specimen from Kota Kota, male with active gonads, 1050 mm. in total length.

This species differs from all other members of the endemic short-gill-rakered group in the width of the vomerine tooth-band, more than twice the relative width of the band of any other endemic species, the bluntness of the teeth and the slimness of the body. The modification of the teeth suggests a molluscan diet, as in the case of *Clarias mellandi* (where the vomerine tooth-band, although also broad, is entirely different in shape), but no molluscan remains were observed in the stomach contents. The stomach of the type contained numerous scales and fin-ray bones of cichlid fish, and three small lumps of charcoal, while the paratype's stomach was empty. Both fishes were taken on long lines, which suggests a markedly carnivorous habit. The species is probably fairly uncommon, as only these three specimens were observed during the course of the survey.

BATHYCLARIAS FILICIBARBIS sp. nov. (Fig. 5)

Depth of body 7 in total length ; head length 3·3 times in standard length ; width of head 4·4 times in standard length, its upper surface smooth and flattened ; occipital process moderately blunt. Frontal fontanelle dagger-shaped, 6·5 in length of head, three times as long as broad ; occipital fontanelle 19·5 times in length of head, twice as long as broad. Eye lateral, 17·9 times in head length, nine times in interorbital width. Width of mouth 1·8 times

in length of head, 1·2 times in interorbital width. Length of snout 3·9 times in head length. Nasal barbels aberrant, cone-shaped, with frilled bifid top, 31 times in length of head ; maxillary barbels broad, strong, flattened, four times as wide as deep, distal third with 13 frilled fleshy lappet-shaped

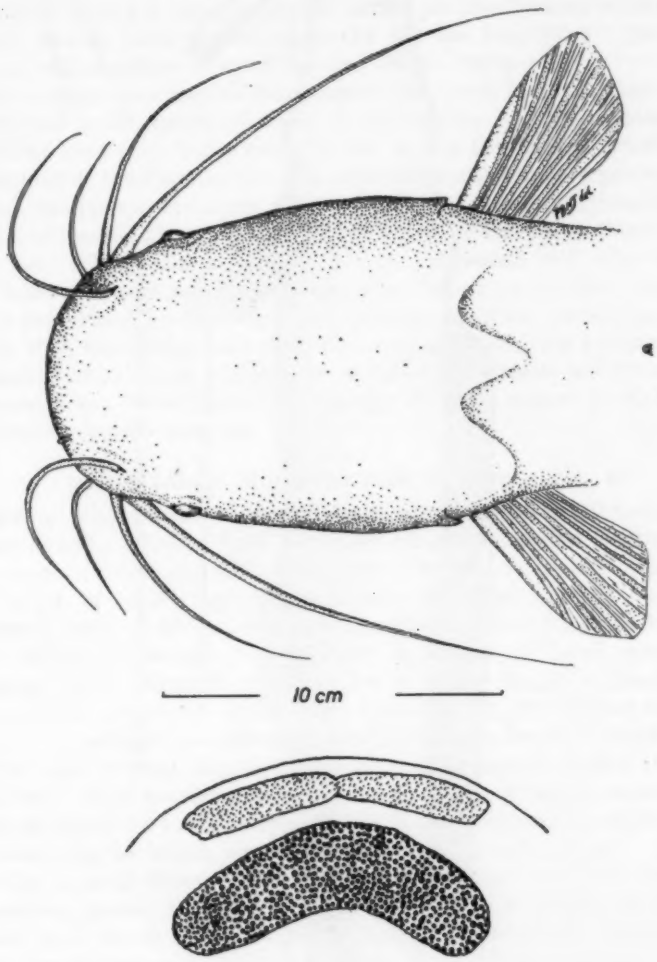

Fig. 4.—*Bathyclarias euryodon* gen., sp. n. Type, male.

proliferations on posterior edge, anterior edge crinkled and frilled, length of barbel 2·1 times in length of head ; outer mandibular barbel similarly modified, 2·8 in length of head ; inner mandibular similar but smaller and weaker, 6·5 in length of head. Half praemaxillary tooth-band 4·6 times in length of head, its width 5·1 times in length of half band ; vomerine tooth-band width 6·5 in

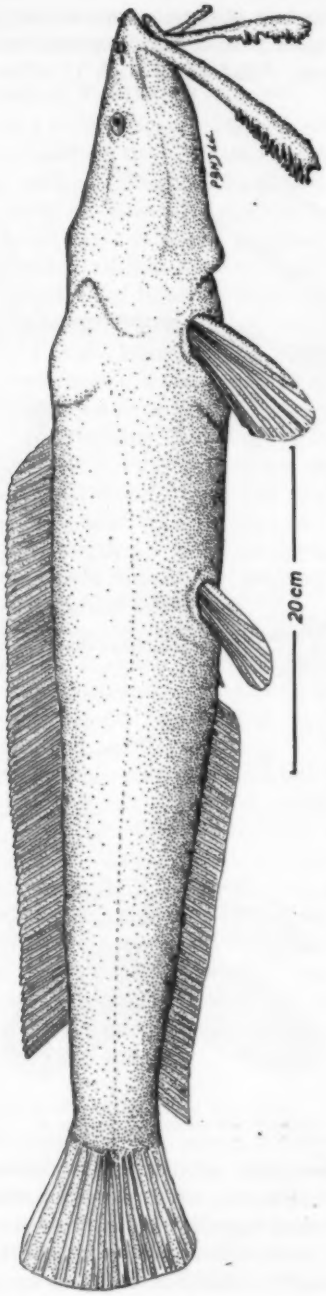

Fig. 5.—*Bathyclarias flicibarbis* gen., sp. n. Type, male.

half length, with a marked gap ; teeth pointed. Gill-rakers very short, about one-third length of filaments, 117 on outer branchial arch. Dorsal 70, its distance from occipital process four times in length of head. Anal 51, well separated from caudal fin. Pelvic fins moderately large, about mid-way along body ; pectoral fin with a strong spine. Colour uniformly deep purplish black, overlaid, in the fresh condition, with a jet black mucus which is readily removed, leaving black smears where the fish has been lying. The holotype a female, with inactive or spent gonads, length 792 mm.

Only a single specimen of this remarkable fish is known, taken by 5-inch mesh gill-net in 55 metres of water off Nkata Bay. Another, however, was reported to have been taken near Nkudzi Bay in the shallow South-east arm by fishermen of the Colonial Development Corporation fishery project in 1952. Although the present specimen was taken in deep water, its stomach contents consisted of two prawns (*Caridina nilotica* Roux) and remains of one very small cichlid fish. The prawn is associated with *Vallisneria* and other vegetation in the lake, and does not occur deeper than about 15 metres. Further, the fish was parasitised by the branchiuran *Dolops ranarum* (Stuhlmann). Fryer (1956, p. 329) has shown that, with this one exception, this parasite has been found in the Lake Nyasa region only on fishes in estuaries and rivers, such as *C. mossambicus*. This evidence, though scanty, argues a shallow-water habitat rather than a deep one.

BATHYCLARIAS WORTHINGTONI sp. nov. (Fig. 6)

Depth of body 6·8 to 7·5 in total length ; length of head 3·3 to 3·5 times in standard length ; width of head 4·3 to 5·0 times in standard length, its upper surface smooth ; occipital process angular. Frontal fontanelle oblong, 4·2 to 5·7 in length of head ; occipital fontanelle 10 to 25 times in head length. Eye lateral, 14·5 to 20 times in head length, 8 to 12 times in interorbital width. Width of mouth 1·7 to twice in length of head, greater than interorbital width. Length of snout 3·4 to 4·4 in length of head. Length of nasal barbels 2·2 to 3·7, of maxillary barbel 1·1 to 1·6, of inner mandibular 2·0 to 2·7, and of outer mandibular 1·3 to 1·8 times in length of head (for fishes above 250 mm. in total length) ; barbels tapering rapidly from a thick fleshy conical base. Half praemaxillary tooth-band length 4·5 to 5·7 times in length of head, its width 3·8 to 6 in length of half band ; vomerine tooth-band width 4·2 to 5·5 times in length of half band, usually a gap present. Gill-rakers 100 to 130 in adult fishes, short (see Table 1). Dorsal 64 to 69, its distance from occipital process 3·4 to 5·2 times in the length of the head ; anal 52 to 56, separated from the caudal by a gap. Caudal fin moderately small and compact, the caudal peduncle longer than deep. Pectoral fin with a strong spine ; pelvic fin about midway along body. Colour green brown or dark brown, with a tendency for a lightening of colour ventrally and a darker marbling over the whole of the body. The lips are often fleshy, and this with the wide base of the maxillary barbel gives a characteristic " hammer-head " appearance when viewed from above which is enhanced by the rapid broadening of the head behind the eye. Maximum recorded length 814 mm.

The holotype a female, with active gonads, 682 mm. in total length ; also

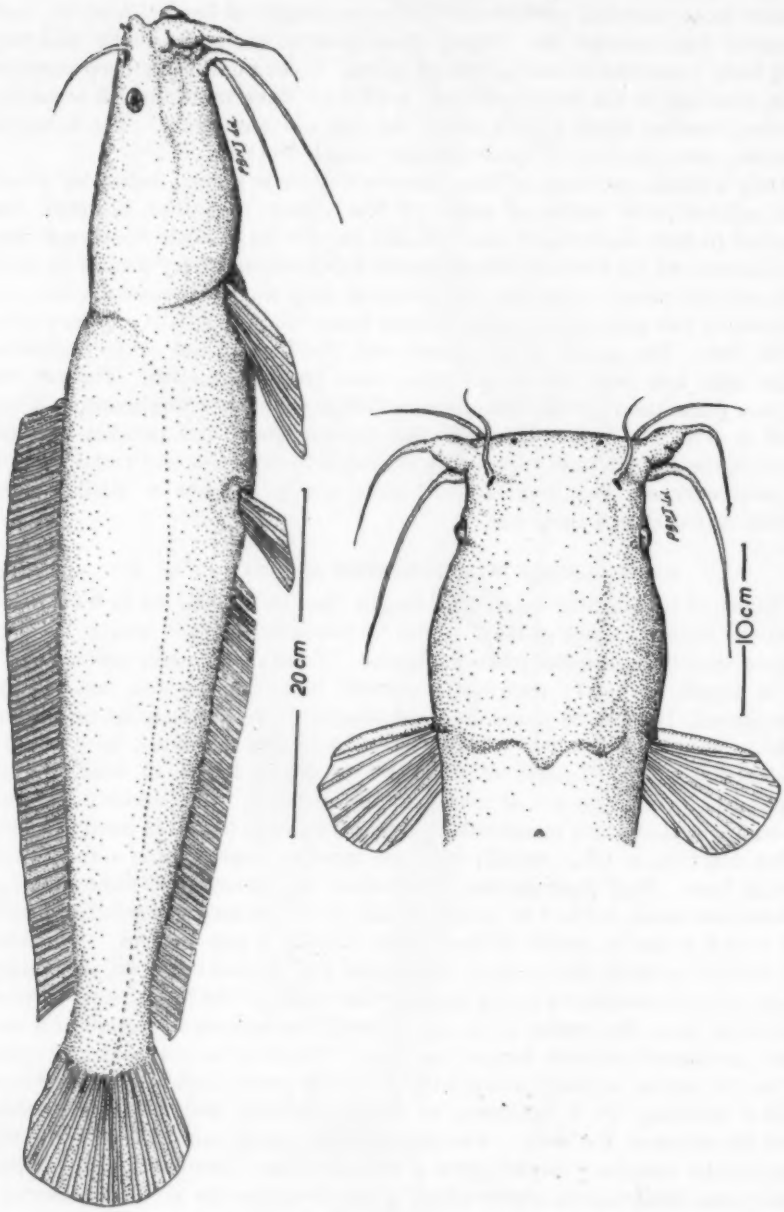

Fig. 6.—*Bathyclarias worthingtoni* gen., sp. n. Type, female.

two paratypes of 275 and 254 mm. deposited in the British Museum, and two juveniles of 75 and 80 mm.

This well-marked species is numerous and wide-spread at Nkata Bay, Likoma Island and elsewhere over the central deep-water areas of Lake Nyasa, among rocks. The diet consists largely of the Lake Crab, *Potamonautes lirrangensis* Rathbun, the size of the crab taken varying with the size of the fish. It occurs at all depths down to at least 40 metres, juveniles being fairly common among rocks in very shallow water. Fryer (1959) gives an account of the part these fishes play in the ecology of the rocky lake littoral at Nkata Bay.

I have pleasure in naming this new species after Dr E. B. Worthington, whose taxonomic studies on the non-cichlid fishes of the Central African region have proved to be such a valuable foundation on which to base future work.

BATHYCLARIAS GIGAS sp. nov. (Fig. 7)

Depth of body about 6·0 to 6·5 times in total length ; length of head 2·6 times in standard length ; width of head four times in standard length, its upper surface flattened and fleshy, without granulations, 1·3 times as long as wide ; occipital process moderately angular. Eye lateral, 18 times in head length, 14 times in interorbital width. Width of mouth about equal to interorbital width, 1·6 times in head length. Length of snout four times in length of head. Length of nasal barbels 6·0 times in length of head, of maxillary barbels 2·3 times, of inner mandibular 4·2 times and of outer mandibular 3·1 times in length of head. Teeth in two broad crescentic bands, pointed, directed backwards ; half praemaxillary tooth-band length 4·7 in head length, width 4·4 times in half length of band ; vomerine teeth without gap, pointed, in a band slightly broader than praemaxillary, crescentic. Gill-rakers short, about half length of filaments, moderately well separated, 149 in total number. Dorsal 69, its distance from occipital process 5·8 times in head length ; anal 56, separated from the caudal by a gap. Caudal fin moderate in size ; caudal peduncle about as long as deep. Pectoral fin with a fairly strong spine ; pelvic fin situated about mid-way along body. Colour uniformly black, somewhat lighter beneath.

The holotype a single specimen, male, 1350 mm. in total length, weight 32·65 kg. gonads inactive, stomach empty.

This species is in all probability the largest in the genus, and in the family is exceeded in size by few, if any species. These giant catfishes are well known to lake Nyasa fishermen, who do not consider this to be an exceptionally large example, but it proved to be very difficult to secure a specimen. Twice during the survey an enormous catfish was entangled in the deep-set gill nets and actually seen when the net was hauled to the surface, only tantalisingly to break free at this juncture. Very large clariids such as this one are known at Nkata Bay as " Ntanda ", but the names " Pwefu " and " Nkopora " are applied also to moderately large catfishes ; as Africans recognise fishes by the colour and general facies, taking no account of characters such as dentition and gill-rakers, these latter names may also apply to large specimens of *B. worthingtoni* and *B. euryodon*, among others, as well as to smaller specimens of *B. gigas*.

KEY TO THE FAMILY CLARIIDAE IN NYASALAND

Excluding that part of the Shire River system lying below the Murchison Cataracts, which has a Lower Zambesi fauna and is not considered here, there are in Nyasaland four members of the genus *Clarias*, each with a wider distribution. These are *Clarias mossambicus*, *C. mellandi*, *C. theodorae* and *C. carsonii*. Only the latter two have the pelvic fins appreciably nearer the end of the snout than the root of the caudal. None of the non-endemics has colonised Lake Nyasa to anything like the same extent as the endemic genus *Bathyclarias*, and *C. carsonii* is found only in cold high upland streams.

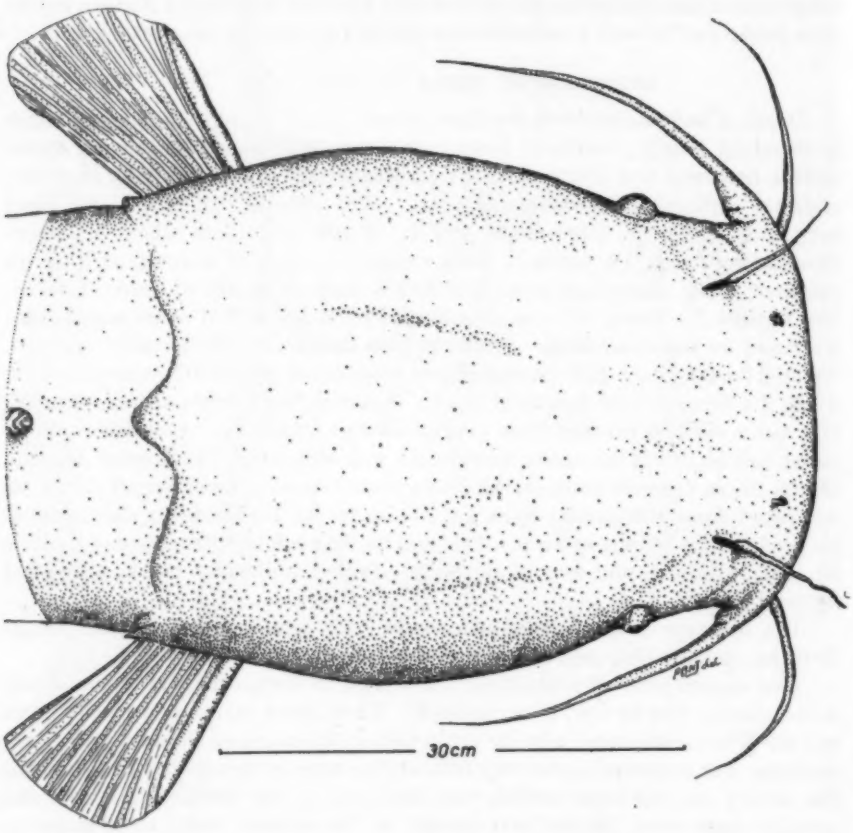

Fig. 7.—*Bathyclarias gigas* gen., sp. n. Type, male.

1.	Eye dorsal, forming no part of head outline when viewed from above	(Genus *Clarias*)	2
	Eye lateral, breaking into head outline from above	(Genus *Bathyclarias*)	5
2.	Pelvic fin appreciably nearer tip of snout than root of caudal		3 (1)
	Pelvic about midway along body		4 (1)

3.	A gap between dorsal and caudal fin greater than diameter of eye	–1– *C. carsonii*	(2)
	Gap between dorsal and caudal absent or very small	–2– *C. theodorae*	(2)
4.	Vomerine teeth forming a semi-elliptical patch	–3– *C. mellandi*	(2)
	Vomerine teeth forming a narrow band	–4– *C. mossambicus*	(2)
5.	Gill-rakers 0·3 to ·6 in length of filaments, usually less than 140 in number		.....6 (1)
	Gill-rakers 0·8 to 1 in length of filaments, usually more than 150		.....12 (1)
6.	Barbels rounded, smooth, simple		.....7 (5)
	Barbels broadly flattened, a series of rounded lappets distally	–1– *B. filicibarbis*	(5)
7.	Body smooth, without pits, barbels moderate to long		.....8 (5)
	Body squat, humped, rugose and pitted	–2– *B. foveolatus*	(5)
8.	Vomerine tooth-band less than 1½ times as broad as praemaxillary ; teeth discrete and pointed		.....9 (7)
	Vomerine teeth more than 1½ times as broad as praemaxillary ; teeth blunt and concrete	–3– *B. euryodon*	(7)
9.	Maxillary barbel not reaching beyond extremity of pectoral fin		.....10 (8)
	Maxillary barbel reaching well beyond extremity of pectoral fin	–4– *B. longibarbis*	(8)
10.	Head flattened above, edge of snout only slightly curved in dorsal outline, snout less than 4·6 times in head length		.....11 (9)
	Head rounded and chubby, snout strongly curved and at least five in head length	–5– *B. rotundifrons*	(9)
11.	Head length more than 3·3 times in standard length ; base of maxillary barbel markedly conical and fleshy ; vomerine toothband narrower or as broad as praemaxillary	–6– *B. worthingtoni*	(10)
	Head less than 3·1 times in standard length ; base of maxillary barbel not conical and fleshy ; vomerine toothband broader than praemaxillary	–7– *B. gigas*	(10)
12.	Body elongate, scarcely humped behind head ; caudal peduncle 1·1–1·4 times as long as deep ; maxillary barbel more than 1·4 in head length		.....13 (5)
	Body massive, markedly humped behind head ; caudal peduncle as deep or deeper than long ; maxillary barbel less than 1·2 in head	–8– *B. loweae*	(5)
13.	Gill filaments red ; no black mucus on body	–9– *B. nyasensis*	(12)
	Gill filaments purplish-black ; black tarry removable mucus on body	–10– *B. ilesi*	(12)

ACKNOWLEDGMENTS

I am very grateful to Dr Ethelwynn Trewavas of the British Museum of Natural History for help and advice while I was working at the Museum ; it was Dr Trewavas who first drew my attention to the lateral position of the eye, found later to be a crucial character in all the Nyasa endemics, in an early specimen of *B. foveolatus*. I am indebted also to Mr P. H. Greenwood of the British Museum for dissecting a paratype of the same species and drawing my attention to the reduction of the suprabranchial organ, found also to occur in all endemic species, and to my colleagues Messrs G. Fryer, D. Harding and T. D. Iles for their help, in particular to Mr Iles who collected many specimens, did a good deal of measuring and offered useful suggestions.

SUMMARY

A study was undertaken of catfishes of the family Clariidae in Nyasaland, excluding that small part of the Shire River system which lies below the Murchison cataracts. Of fourteen species found, four, belonging to the genus *Clarias*, are not endemic and are found mainly in rivers, streams and swamps, inhabiting Lake Nyasa sparsely and only where similar conditions are found. The lake has been colonised mainly by the remaining ten species, which are all endemic to it, specialised for lacustrine conditions and sufficiently distinctive to warrant a new genus being proposed : genus *Bathyclarias* nov. The ten species of the genus form a species-flock, having a variety of habitats. Seven are new and are described and figured : *Bathyclarias loweae*, *B. ilesi*, *B. rotundifrons*, *B. euryodon*, *B. filicibarbis*, *B. worthingtoni* and *B. gigas*, spp. n. Notes on distribution and ecology are given in all cases, and a key is given for all members of the family in Nyasaland.

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REPORT OF THE SOCIETY'S PATHOLOGIST FOR THE YEAR 1957

BY

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[Accepted 14th October 1958]

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INTRODUCTION

For the past six years, pathological reports have appeared under the signature of the Society's Prosector, Dr W. C. Osman Hill, who assumed the responsibility from Dr R. E. Rewell in 1950.

During the year under review, the present author became responsible for the pathology work when the Society's new Hospital and Pathology Laboratories were completed on January 1st. Dr Hill remained in charge of the Prosectorium and is thus able to devote his entire time to anatomical research. The Pathologist, meanwhile, is able to provide an improved diagnostic service for the Veterinary Officer ; in addition, proper research and extension work, as and when found necessary, can be more satisfactorily undertaken.

The work will now be considered under three main headings, firstly Diagnostic Section, secondly, Autopsy Examinations, and thirdly, Research.

DIAGNOSTIC SECTION

The positive results from 457 general laboratory examinations are detailed in Appendix 1 and from 588 bacteriological examinations in Appendix 2. The majority of the laboratory investigations undertaken consist of the examination of faeces for worm eggs and other parasites and skins for mange, etc. Special mention may, however, be made of certain problems as follows :—

1. *Bacteriological testing*

With the increased use of antibiotics and sulfa drugs, it has been found that organisms isolated either from stool specimens or from sputum are often resistant to the commonly used drugs. For this reason, it has become standard practice to select antibiotics or sulfa by means of cultures made on sensitivity plates ; otherwise these drugs are rarely used. Whatever the theoretical argu-

ment against the validity of *in vitro* sensitivity testing of organisms, this practice has abundantly justified itself and resulted in the saving of the lives of many animals which would have died. An instance of this was the case of six young chimpanzees, recently imported from Holland. One week after being placed on exhibition, all the chimpanzees developed pneumonia and pneumococci were isolated from sputum samples. Antibiotic treatment had no effect on the condition, and sensitivity plates were prepared. The pneumococci proved to be solidly resistant to all the antiobiotics tested including penicillin, streptomycine and the tetracyclines. It was, however, found that the organisms were sensitive to sulfa and the pneumonia was rapidly cured by injections of sulfathiozole, a drug which would not normally have been used.

2. *The diagnosis of enteric organisms*

Salmonellosis has only been infrequently encountered during the year under review. In the birds, a good deal of trouble is experienced from gut infections, many of which have proved to be associated with *Bacillus coli*. More will be said of this in the Research section. Our main trouble, however, has been amongst the Primates from infection with organisms of the dysentery sub-group, in particular *Shigella flexneri* 103, *Shigella flexneri Newcastle*, *Shigella sonnei* and, more rarely, *Shigella schmitzii*. Infection with one of these organisms is continually appearing among animals in the Monkey House and, once it has appeared, passes rapidly from one end of the House to the other. Treatment is not always effective and sometimes repeated stool samples are sent to the laboratory for examination. Following treatment, some of the monkeys appear to become carriers, and may re-start the infection after it has been thought to have been overcome. Some monkeys continually return for treatment because of recurrent ill health and, although infected, the organism cannot be isolated from the stool samples.

3. *Tuberculosis*

The dangers of tuberculosis cause concern in all groups of animals, but in particular amongst the monkeys. The disease is, however, also encountered amongst the Artiodactyla and amongst birds. Early diagnosis is essential, and presents certain difficulties in a laboratory such as this where animals are not used for diagnostic purposes. In spite of this, some success has been achieved by cultural methods as follows. We normally make our diagnoses of tuberculosis from stool samples which are digested in antiformin overnight in the incubator, centrifuged to concentrate the residue which is then examined for acid-fast organisms. A similar method can be used for purposes of culture, and the residue is seeded on to a modified Dubos medium, as well as Loewenstein, and this gives a very rapid growth of mycobacterium which can then be seeded on to glycerine-containing medium to determine whether it is dysgonic or eugonic. Normally, of course, the eugonic organisms will be of human type, and the dysgonic of bovine type. In the cases of avian tuberculosis, organisms are usually present in such large numbers as to present no difficulty.

AUTOPSY EXAMINATIONS

Tables showing the deaths and causes of deaths and other information are set out in the usual form, Appendices 3 to 5. The various headings which cover causes of death will now be considered in detail.

1. *Inanition*

Snakes and chameleons often die from inanition during the early months of captivity when they refuse to eat. After the first six months less trouble is encountered from this cause. There is some reason to think that in snakes this may sometimes be associated with infection of the blood with haemogregarines. More is said of this later under the section Research. The death of a female Canada Goose was recorded as being due to inanition since this bird appears to have died from starvation due to refusal to feed following the death of its mate.

2. *Injury and/or accident*

Losses from injury and accident are especially common during the months April to June, that is during the breeding season. At this season there is much fighting especially amongst the birds and deaths and injuries are common.

Severe losses from this cause were experienced amongst a consignment of iguanas from South America. These creatures are apparently offered for sale, tied by the hind legs, and suspended from hooks. Nineteen arrived in the Gardens with gangrenous legs and seven died from septicaemia from this cause. A further five of the same consignment are recorded as dying from the retention of necrotic ova also, no doubt, the result of harsh treatment. Cultures of the infecting organisms revealed the presence of *Clostridium welchii*, an anaerobic streptococcus and a gram-negative bacillus, possibly *Proteus* sp.. All these organisms proved penicillin resistant, but sensitive to aureomycin. The remaining iguanas recovered following treatment.

3. *Acute specific fevers*

Ornithosis has been virtually absent from the Gardens during the entire year. No anxiety has been experienced from any form of epidemic disease.

4. *Tuberculosis*

Sporadic deaths have occurred amongst the monkeys, antelopes and birds. The disease, however, appears to be well under control and there is at present no epidemic.

5. *Mycosis*

(a) Aspergillosis of the lungs and air sacs takes a heavy toll of the newly imported penguins, especially the King penguins. Three newly imported birds were lost from this cause during the year though their susceptibility to infection may have been influenced by the fact that they had never started to feed in captivity.

(b) Actinomycosis in kangaroos (due to *Actinomyces macropodidarum*) continues to be troublesome and was diagnosed in a Bennett's Wallaby and a Red Kangaroo.

A new and disturbing circumstance during the year has been the death of three Fishing cats from generalised actinomycosis due to *Actinomyces* sp. (probably *A. Israeli*). The condition has arisen in tongue and jaw and may affect also the lungs and alimentary tract. Attempts at treatment have not been successful.

6. *Diseases due to parasites*

Nematode and cestode infections continue to be troublesome in the Gardens in spite of frequent treatment ; deaths recorded from these causes are not numerous. The mortality amongst snakes from nematodes and amoeba has been reduced to negligible proportions following action to overcome them, reported under the heading of Research.

7. *Diseases of the blood and circulatory organs*

Most of the hearts from animals dying in the Gardens during the year have been examined by Dr C. Symons, a Cardiologist attached to the Royal Free Hospital, Hampstead. Amongst other examinations he has particularly examined the coronary arteries. Very little evidence of disease has, however, been discovered amongst any animals. Apart from these studies, no cases have occurred of sufficient importance to report separately.

8. *Diseases of the alimentary system*

Enteric disease is a serious cause of loss both amongst the mammals and amongst the birds. Some studies on the causes of the alimentary diseases amongst the birds will be described under the heading of Research.

Amongst the mammals, the dysentery sub-group of organisms is of greatest importance. Losses from this cause during the year included a young Polar Bear, a Brazilian Tree Porcupine, and two Soay Sheep. Amongst the birds a shag died of enteritis associated with a Sonnë dysentery organism.

9. *Diseases of the respiratory system*

Sporadic deaths from pneumonia and other respiratory diseases have occurred throughout the Gardens but there has been no form of epidemic. Infectious coryza quite commonly occurs amongst all classes of birds.

10. *Diseases of the urinary organs*

Diseases of the urinary organs as a cause of death have been comparatively rare. Chronic nephritis is commonly associated in the birds with senility and it appears may be a predisposing cause of other infections.

11. *Diseases of the generative organs*

The commonest diseases of the generative organs are ruptured oviduct or retained ova in birds and reptiles. Cases are especially frequent during the spring. There has already been mention of trouble in some iguanas from South America.

12. *Diseases of the nervous system*

There is virtually nothing to report under this heading. A single case occurred of meningitis associated with the presence of meningococcus in a galago.

13. *Diseases of metabolism*

The case of supposed metabolic disease amongst young lions will be considered under the heading of Research. Meanwhile, a number of apparent rickets cases have been encountered in New World monkeys and hedgehogs.

14. *Neoplasms*

There is nothing to report under this head.

Cases of special interest

The following cases of exceptional interest are worthy of individual mention :

Mammalia

Polar Bear. Died from acute enteritis. A dysentery bacillus of the Flexner group was isolated from the gut contents.

Brazilian Tree Porcupine. Died from haemorrhagic enteritis, a Flexner dysentery bacillus was isolated which was agglutinated by the serum of the last case, the Polar Bear.

Monteiro's Galago. Died of meningitis. Meningococcus and *Corynebacterium pyogenes* were isolated.

Elk. This elk was affected with clinical paratyphoid and a typical gut ulceration was seen on post mortem. He appears to have been recovering from this. Organisms could not be isolated from his gut but his serum agglutinated *Salmonella paratyphosum C* in high dilution. His peritoneum was heavily infested with bladders of *Cysticercus tenuicollis* one of which lay on an exposed portion of the portal vein and between it and the ribs. The elk died of typical symptoms of portal obstruction which must have been caused by pressure from the tapeworm bladder ; there were some gallons of fluid in the abdominal cavity and the blood showed a corresponding haemo-concentration due to anhydraemia. A case somewhat similar in a snake is described below.

Serval Cat. This case was a young serval kitten which was affected with a diaphragmatic hernia. The hernia was believed to be congenital since the stomach had passed forward into the thorax through the oesophageal groove of the diaphragm.

Two Soay Sheep from the Mappin Terraces. Two Soay sheep were found dead without history of illness. Both carcases showed signs of a septicaemic condition with enteritis, peritonitis and nephritis. Cultures from both carcases revealed the presence of *Shigella flexneri* 6. No other pathogenic organism was isolated. Six days later this same organism was cultured from the stool of a Spider monkey in the Monkey House. The septicaemic death of the two Soay sheep presumably from this infection is most unusual. It seems probable that the infection derived from some visitor to the Zoo who visited both Monkey House and Mappin Terraces. Enquiries have revealed no other source of physical contact betwen the two places and no case of dysentery or diarrhoea was reported amongst the Society's staff.

The determination of this organism as *Shigella flexneri* 6 was confirmed by the Public Health Laboratory at Colindale. This is believed to be the first report either of infection or of supposed deaths of sheep from dysentery sub-group organisms.

Deaths of Saiga Antelopes. Four Saiga antelopes died during the year and were examined. The difficulties encountered over maintaining these antelopes in captivity are well known and the findings at post mortem are, therefore, of interest. All the antelopes died from different causes with no common denominator. The results were as under :—

(a) *Fully grown female.* Died of septicaemia due to infection with *Clostridium welchii*, a typical case of the pulpy kidney type.

(b) *Young male.* This young Saiga was infected with typical foot rot as seen in sheep. The causal organism *Fusiformis* sp. was isolated from it. The foot responded to treatment but the organism had gained entry to the body and set up liver abscesses from which *Fusiformis* was again cultured.

(c) *Full grown female.* Death in this case was due to septicaemia with acute nephritis. The only organism isolated and present in the kidneys in very large numbers was *Bacillus anthracoides*. This organism would normally be regarded as non-pathogenic but it is occasionally found in zoo animals under circumstances which give rise to the suspicion that it may at times cause disease.

(d) *Young female.* This young female was destroyed because of partial paraplegia. She suffered from extensive crush fractures of the lumbar vertebrae. It is supposed that the heavy male Saiga may have caused these in attempting to mount her.

Three Fishing Cats. As already mentioned, these Fishing cats died from actinomycosis. The infection was found in tongue and mandible, also in the lungs of two of the cats, and in one case there was severe involvement of the intestines. The organism was anaeorobic and thus Actinomyces not Nocardia.

Tiger "Rimba". A fine male tiger, "Rimba" was lost from miliary tuberculosis following a peculiar medical history. He was admitted to Hospital because of lameness in one stifle joint. After return to the Lion House, he rapidly became emaciated and eventually had to be destroyed. The lesion which was present in both stifle joints was a simple arthritis, and although the joint was full of pus, it was not affected with tuberculosis. It is supposed that he became infected with tuberculosis partly as a result of fretting at his injury.

Tiger "Warrior". This tiger was supposed to be a tuberculous case but laboratory examination had persistently failed to reveal the presence of infection. He was eventually destroyed because of progressive emaciation. Post mortem revealed that he was affected with acute pulmonary and gastric nematodiasis. There was severe bronchitis due to the presence of numbers of larval nematodes which it was not possible to identify. In the small intestine were large numbers of the hookworm *Ancylostoma ceylanicum* and both intestines also contained numerous nodules in a sub-capsular position in which were found further hookworms known as *Galoncus perniciosus*. The tiger showed all the typical signs of severe hookworm anaemia.

Manatee. A strange case occurred in one of the manatees, which died suddenly. Death occurred following a week of apparent nymphomania during which the animal was continually attempting to copulate with his mate. On opening the abdomen, a gallon or more of clear, dark yellow urine was found. At the same time the thorax was filled with blood. The blood in the thorax

was found to be due to an irregular tear in the right auricle. This was plainly the immediate cause of death. The animal was suffering from an acute septicaemic nephritis from which *Bacillus pyocyaneus* was isolated in pure culture. The dorsal wall of the pelvis contained nothing but a jelly-like mass of myxomatous tissue resembling Wharton's jelly. The two epididymides were identified together with the two vasa deferentia. There was, however, no trace of testes or of the other accessory sex glands. It can only be assumed that in addition to the acute nephritis, there was an acute orchitis with stimulation of libido and that the testes ruptured and disintegrated into a post-inflammatory myxomatous mass.

Aves

Canada Goose. A Canada goose died from failure to feed about two weeks after the death of her mate.

Black-footed Penguin. This was a very thin bird and much debilitated. It suffered from hyperkeratosis of the beak which more or less blocked the external nares. The penguin suffered from sinusitis and there was pus in the eustachian tube. The pus gave a pure culture of *Clostridium welchii* which may have been the cause of the condition.

Reptilia

Boa Constrictor. The death of this boa constrictor was due to an old crush injury of the body wall which had led to stricture of the oesophagus and inability to swallow.

Puff Adder. This snake had a ruptured gut consequent on eating its companion of the same species. The companion was half an inch longer than itself.

Crossed Viper. This snake died of necrosis of the lower gut. Mention is made of it for purpose of reporting an enlarged thyroid which proved to be cystic.

Indian Python. The death of this python was of some interest. It was moderately infested with nematodes in stomach and duodenum. As usual in the stomach the worms had gnawed into the mucous membrane causing abscess formation. The abscesses had partly organised into a fibro-necrotic mass. This mass was exerting pressure on the long cystic duct leading from the liver to the pancreas and duodenum. The damming back of the bile had caused a fatal necrosis of the liver which was, however, not accompanied by ascites.

RESEARCH

General work

During the year a card index of the literature on tumours in wild animals was compiled. The incidence of connective tissue and epithelial tumours amongst captive animals, and the organs affected in order of frequency is tabulated below.

	Connective tissue Percentage	Epithelial Percentage
Artiodactyla	50	40
Carnivora	15	80
Primates	33	50
Rodentia	40	60

	Percentage		Percentage
Kidneys	10	Intestines	5
Bones	9	Lungs	4
Thyroid	9	Pancreas	4
Liver	8	Stomach	4
Nose, mouth, larynx and pharynx	6	Serous membs.	3
Skin and scalp	5	Testicles	3
		Other organs	22

The figures in the first table may be of interest as showing a possible correlation between feeding habits and type of tumour developed. In the second table, the high incidence of thyroid cancer is due to the unusual susceptibility of certain Carnivora, especially the Canidae, and of certain Rodentia. The high incidence in the kidneys of this kind of tumour is due to the special susceptibility of birds to tumours in this site.

Diseases of snakes

The mortality amongst snakes at the Regent's Park Gardens was inclined to be unduly high and some research was undertaken on the subject. The main diseases causing death are three in number :

(i) Inanition. Newly imported snakes often refuse food and eventually died of anaemia and inanition. The possibility that this was due to infection of the blood with haemogregarines or haemoproteus was studied. Blood smears were examined from sick and healthy snakes and from snakes brought in for post mortem. The result of these examinations showed :—

(a) Healthy snakes may carry a heavy infection with haemogregarines which cause no apparent symptoms.

(b) Such heavy infections do not diminish with treatment by Daraprim.

(c) Most healthy snakes in the collection are not infected.

(d) Snakes which have died of anaemia and inanition have been infected in all cases examined.

It does, therefore, appear that while these parasites may be non-pathogenic, they may also be in part the cause of the anaemic condition so commonly found in newly imported snakes.

(ii) *Amoebiasis.* Snakes at post mortem are commonly found to be infected with *Entamoeba invadens.* This parasite causes extreme ulceration of the colon in the terminal portion where the gut and kidney are linked by perito-

neum. Inflammation sometimes spreads from the gut to the kidney. Many fatalities occur from amoebiasis and no satisfactory methods either of diagnosis or of treatment existed.

The difficulty over diagnosis rested on the absence at most times of amoebic cysts from stool, cloacal swabs and gut scrapings. It was found that in infected snakes, cysts could always be recovered from the liver at post mortem although this organ did not appear to be in any way damaged by the amoeba. Some work was done to ascertain whether liver puncture could be used as a method of diagnosis. Liver punctures made on dead snakes were on several occasions, successful in demonstrating the presence of cysts. This work, was, however, not extended to living specimens although it is to be recommended as a post mortem technique.

Dr Neal of the Wellcome Institute became interested in our problem and was able to infect grass snakes artificially with cysts of *Entamoeba invadens* and demonstrated that infection could be certainly diagnosed, even when cysts could not be found in either gut or liver, by cultural methods.

Since then, all snakes in the Reptile House have been tested by faecal culture for amoebae. As a result, two snakes have been found which carry infection although they show no symptoms and are in good condition. Meanwhile, measures have been taken in the Reptile House to prevent the spread of this infection and amoebiasis in the snakes has now ceased to be a problem.

(iii) *Nematodiasis.* The third major disease as a cause of death in snakes is infestation with nematodes, especially *Polydelphis anoura.* In these cases, the adult worms are found either in the stomach or else in the proximal portion of the duodenum. The numbers in the stomach are usually small but it is in this organ that the trouble occurs. The worms burrow into the mucous membrane, gnawing their way through to the muscular layers. The effect is serious ; abscesses develop which run between the muscular and sub-mucosal layers of the stomach wall causing widespread separation between the two. Often the stomach wall may become thickened to three or four inches.

Infection with this worm is easily diagnosed since the ova may be found in the faeces. Following some deaths of valuable pythons, faecal samples from all snakes were examined for infection and those which were found infected were treated by the Veterinary Officer. Repeat examinations after treatment showed that further treatment required to be given with every feed over a matter of months. Eventually, however, the snakes were cleared and deaths from this cause are now negligible.

A number of snakes are sometimes lost also from the presence of the worm *Rhabdias* in the lungs. In cases of infection, the surface of the lungs becomes coated with greyish pus in which the small larval-like worms and their eggs are very numerous. The treatment given by the Veterinary Officer for other nematodes has, it is thought, had some effect in this condition since deaths from pulmonary nematodiasis have been reduced to insignificant proportions.

4. *Bacterial flora of the intestines of some groups of birds*

A study of the normal bacterial flora of the intestines of birds has been made by Mrs Honigmann. Losses amongst the birds from various forms of enteritis

have been severe in recent years. Bacteriological examination of the gut of birds lost from enteritis aroused the suspicion that many cases were due to infection with *Bacillus coli* and that in certain species *B. coli* was not a normal inhabitant of the intestines. It was decided, therefore, to examine the normal bacterial flora in the excreta and intestinal contents of different groups of birds.

The results obtained so far are shown in Appendix 6. This shows that in certain birds, namely the psittacines and the humming birds, gram-negative bacilli are absent from the normal gut. In the case of the main groups of seed eating birds such as the finches this flora is mainly gram-positive but a few *B. coli* may be present. A culture plate showing massive gram-negatives indicates a pathological gut.

Wild starlings and sparrows were shot in the Gardens and the guts of the starlings were found to contain *Bacillus coli*, the guts of the sparrows usually contained no gram-negative bacilli. If present, they were very scanty. After leaving the bodies of these birds in the incubator for up to twelve hours, gram-negative bacilli did not appear except in small numbers.

The problem of enteritis in captive wild birds appears to be very complex. The main form taken by enteritis cases appears to be a liquefactive necrosis of the upper portion of the small intestine, chiefly the pancreatic loop and involving the pancreas in some inflammation. This we have come to call the " Sloppy Gut " condition and our conclusions in regard to its aetiology may be summarized as follows :—

1. This condition occurs in those birds in which there is normally a gram-positive, or chiefly gram-positive gut flora ; it may be due to the presence of *Bacillus coli*.

2. In other cases, the gut contents have been completely sterile even where it has been known that no antibiotic has ever been used on the birds. In such cases, it is suspected that some virus is involved. Material has been under study at the University College Hospital by Dr Belyavin and preliminary work suggests that a virus will be isolated.

3. In cases of enteritis of the small intestine, careful examination of the upper respiratory passages should be made since it appears often to accompany some forms of Coryza.

In all these cases, there is a severe enteritis which starts as stated at the pancreatic loop of the small intestine and may spread downwards to affect the entire gut. The enteritis takes the form of a liquefactive necrosis and the guts appear sloppy and moist. Attempts have been made to reproduce the sloppy appearance of the gut in the experiments already described with the sparrows and starlings. In these cases, however, in spite of prolonged incubation at 37°C, the guts do not develop the typical sloppy appearance associated with these diseases. It does not, therefore, appear to be associated with pancreatic ferments.

The disease appears to occur as a breakdown of premunity rather than as a new infection at least in so far as the suspected virus form is concerned. In one case, six birds of the finch family were lost from this cause when they

were transferred to a new cage while their own cage was being redecorated. All six died ; all six showed the typical " Sloppy Gut " symptom and none of these birds produced any growth on culture media from the gut contents.

In the case of the form supposed to be associated with *Bacillis coli* this is believed to be the normal *Bacillus coli* present in mammalian and human faeces. Certainly following steps taken to improve the hygiene in the Bird House deaths from this cause have fallen to small proportions.

5. *Paralytic disease of young lions*

In collaboration with the Veterinary Officer an investigation has been conducted into a disease of young lions which appears to be common in these animals when kept in captivity. The disease develops shortly after weaning and takes the form of weakness and instability particularly in the hind limbs, altered gait, peculiar posture and increased irritability. A report on this work will be published in detail elsewhere.

The disease was originally thought to be a myopathy but it has now been ascertained that the muscles are normal and that the peripheral nerves are not affected. The main underlying pathological condition is one of the skeleton and the main lesion an osteoporosis. Examination of the nervous system has revealed that some demyelinating changes occur in the spinal cord and cerebellum. The nature of the changes in the nervous system do not suggest that the paralysis is post-infectious.

The condition is probably of dietary origin and associated with imbalance of the mineral intake.

CONCLUSION

It is expected that in future these reports will take a somewhat different form from the present one, which is prepared on traditional lines. The old system of recording has now been replaced by a punch card system and this will permit of a more detailed analysis of results. It should thus be possible to give a brief account of *all* cases studied.

The cases investigated in the Pathological Laboratory at a zoological gardens have clearly a value out of proportion to their numbers ; indeed, the rarity of the material examined enhances its value, and it is regarded of prime importance that a brief mention of each case should be available to comparative pathologists. Conditions are continually being encountered, which have no exact—and in some cases no—counterpart in either human or veterinary medicine. Certain organisms also, which play little part in human or veterinary pathology, can be very fatal to some species of animals. An attempt will, therefore, be made in future both to establish diagnosis with the greatest possible precision and to publish records, as fully as possible.

The identification of parasites and the accuracy of diagnosis is greatly assisted by the number of scientists, to whom our work is of interest and they assist in their specialised fields. Such services are normally only sought on a reciprocal basis ; a scientist wishing to receive material for study, being requested to send in return a report on the material for the laboratory records.

In this way, some fifteen persons have been provided with material and have in return given expert advice. In addition to these arrangements, diagnostic aids have been afforded by the following :

Dr K. P. Carpenter—identification of organisms of the dysentery sub-group.

Professor R. Lovell—identification of certain obscure bacteria.

Dr M. A. Soltys—typing of acid-fast bacilli.

Dr Mares, and Dr Marion Smith and Dr Walton have assisted in the investigation of the paralytic disease of young lions.

To whom I have to record my gratitude and my appreciation of the valuable advice they have afforded.

APPENDIX 1

Clinical Examinations during the year 1957

Parasites	Host	Nos. Positive
Ankylostoma	Cheetah	1
	Clouded Leopard	1
	Chimpanzee	1
		3
Ascaris	Tiger	6
	Lion	6
	Cheetah	3
	Clouded Leopard	1
	Reticulated Python	1
	Dingo	1
	Maned Wolf	1
	Syrian Bears	2
	Sand Cat	1
	Chimpanzee	2
	Elephant Seal	1
	Leopard	1
	Wild Boar	1
		27
Capillaria and Heterakis	Pheasantry	
	Cages 9, 13, 14, 32	numerous
Cestoda	Cheetah	2
	Clouded Leopard	1
	Reticulated Python	1
	Vulture	1
	Lion	1
	Tiger	1
		7
Oxyuris	Chimpanzee	1
	Orang Utan	1
		2

Strongyloides	Elk	1	
	Tiger	2	
	Orang Utan	1	
	Lar Gibbon	1	
			5
Trichuris	Lar Gibbon	1	
	Chimpanzee	1	
			2
Sarcoptes and Cnemidocoptes	Orang Utan	1	
	Golden Hamster	1	
	Budgerigar	1	
			3
Balantidium coli	Pig-tailed Monkey	1	
	Owl-faced Monkey	1	
	Orang Utan	1	
			3
Coccidia	Big-horned Sheep	1	
	Gerenuk	1	
			2
Troglodytella	Chimpanzee	1	
Trichomonas	Red-handed Tamarin	1	
Actinomyces and other Fungi	Fishing Cat	1	
	Red Kangaroo	1	
	King Penguin	1	
	Yellow Toad	1	
	Corn Toad	1	
Trichophyton	Donkey	2	
			9
	Total		63

APPENDIX 2

Bacteriological and Serological Tests

Table 1. Synopsis of Cultures

Mammalia	319
Aves	246
Reptilia	28
Total	593

Table 2. Enteric Cases

SHIGELLA POSITIVES

A. *Shigella flexneri VI (Newcastle)*

Chimpanzees	3
Spider Monkey	1
Soay Sheep	2
	6

B. *Shigella schmitzii*

Spider Monkey	1
Orang Utan	1
Chimpanzee	1
	3

C. *Shigella sonnei*

Chimpanzees	2
Gibbons	2
Orang Utan	1
Spider Monkey	2
White nosed Monkey	2
	9

D. *Shigella flexneri* 103

Gibbons	2
	2
Total	20

Table 2. Enteric Diseases

SALMONELLA POSITIVES

A. *Salmonella typhimurium*

Kenya Spiny Mouse	1
Canary	1
Homo sapiens	1
Cuscus	1
Silver Pheasant	1
Tiger	1
Cheetah	1
	7

B. *Salmonella anatis*

White-necked Picathartes	2
	2

C. *Salmonella enteritidis*

Wolf	1
Giant Malay Squirrel	2
	3

D. *Salmonella senftenberg (taksony group)*

Swans	2
	2
Total	14

ABERRANT OR INTERMEDIATE ORGANISMS, *of which the pathogenicity is uncertain*

The following organisms have also been found associated with enteric disease in various animals, but the significance is not clear :—

(i) Providence, (ii) *Shigella alkalescens*, (iii) Para-shiga, (iv) Hafnia, (v) Arizona, (vi) Paracolon, (vii) Proteus, (viii) *Bacillus coli*, (ix) *Pseudomonas aeruginosa*.

ANAEROBIC INFECTIONS (Anaerobes according to our experience are not normally present in the guts of predominantly seed—and fruit-eating birds)

A. *Clostridium welchii*		
Canaries		2
B. *Clostridium septicum*		
Honey Birds		1
	Total	3

Table 3. Pasteurellosis

Lop-eared Rabbit	— from abscess	1
Black Rabbit	— „ „	1
Saiga Antelope	— „ lungs	2
Maxwell's Duiker	— „ faeces	1
Bantam Chicken	— pus from mouth	1
Gough Island Coot	— from lungs	1
	Total	7

Table 4. Respiratory Infections

A. *Pneumococcus*		
European Hedgehogs		2
Chimpanzees		5
Bushbuck		1
Saiga Antelope (with Fusiformis and Pasteurella)		2
		10
B. *Klebsiella* (*Pneumobacillus of Friedlaender*)		
Hamster		1
Red Kangaroo		1
Tovi Parrakeet		1
		3
C. *Pneumococcus and Pneumobacillus* (*mixed*)		
Dark-handed Gibbon		1
		1
D. *Haemophilus*		
Chimpanzee (H. influenzae)		1
Tovi Parrakeet (from the brain)		1
		2
	Total	16

Table 5. Mycosis and Actinomycosis

A.	*Aspergillus*		
	Old Rabbit (English) (from lungs)	1	
	Amazon Parrot	1	
	Penguins	5	
			7
B.	*Actynomyces macropodidarum* (*Nocardiosis*)		
	Bennett's Wallaby	1	
	Red Kangaroo	2	
			3
C.	*Actinomyces sp.* (*A. israeli* ?)		
	Fishing Cats	2	
			2
D.	*Monilia albicans*		
	Woolly Monkey	1	
	Chimpanzee	1	
			2
E.	*Monilia batrachi*		
	Corn Toad	1	
	South American Toad	1	
			2
	Total		16

Table 6. Acid Fast Bacilli

Acid fast bacilli were isolated from :—

Woolly Monkey	1	Tigers	3	Cheetahs	2
Capybara	1	Thomson's Gazelle	1	Eland	1
Coypu rat	1	Crab-eating fox	1	Rufous pigeon	1
Canary	1				
				Total	13

Table 7. Cerebral Infections

A.	*Meningococcus*		
	Montoiro's Galago	1	
			1
B.	*Erysipelothrix rhusiopathiae*		
	Birds of Paradise	2	
			2
	Total		3

APPENDIX 3

Necropsies

A total of 798 post mortem examinations was carried out during the year, a decrease of 146 on the previous year. The number is made up of the following categories :—

	Mammals	Birds	Reptiles and Amphibians
1st Quarter	39	77	70
2nd Quarter	33	114	55
3rd Quarter	46	98	88
4th Quarter	44	63	71
Total	162	352	284

APPENDIX 4

	Average strength of stock	Total autopsies	Ratio of total	Animals under six months in Gardens	Ratio of death under six months in Gardens
Mammals	811	162	20·1	50	6·2
Birds	1665	352	21·1	70	4·2
Reptiles and Amphibians	515	284	55·1	99	19·2
Total	2991	798	26·6	219	7·3

APPENDIX 5

Causes of Death

In the table below, both primary and secondary causes of death are registered

	Mammals	Birds	Reptiles and Amphibians	Total
1. Senility	5	23	2	30
2. Inanition	1	5	43	49
3. Injury and/or accident	29	64	20	113
4. Acute specific fevers (incl. Septicaemia, Pyaemia, Cellulitis, virus diseases, toxaemia)	24	20	22	66
5. Tuberculosis	13	10	0	23
6. Mycosis	3	12	2	17
7. Diseases due to Animal Parasites	4	11	13	28
8. Diseases of the blood and circulatory organs	11	29	5	45
9. Diseases of the alimentary system	44	141	42	227
10. Diseases of the respiratory system (excl. mycosis)	31	52	28	111
11. Diseases of the urinary organs	7	54	17	78
12. Diseases of the Generative organs	2	16	16	34
13. Diseases of the locomotor apparatus	3	1	0	4
14. Diseases of the nervous system	3	3	0	6
15. Diseases of the endocrine organs	1	0	1	2
16. Diseases of the pancreas	1	2	0	3
17. Disorders of metabolism (incl. deficiency diseases)	9	6	3	18
18. Neoplasms	2	10	2	14
19. K.B.O.	16	9	0	25
20. Not ascertained (decomposed, not received, reserved for anatomy or food)	4	6	80	90

APPENDIX 6

Results on the findings of the normal intestinal flora in some groups of birds.
Material : Ninety-eight faecal specimens from birds in apparent good health.

Order	Number of specimens	Gram-positive flora	Gram-neg. flora
I Passeriformes	46	prevalent (Micro-and gram-positive diplococci mainly, generally bile-inhibited, fungi, yeast, anthracoids) spec. unknown	in a number of specimens none or few coliform only, also moderate numbers of N.L.F. mostly *B. proteus*
IV Piciformes	15	no dominant factor organisms as above	coliforms fairly numerous
V Coccygiformes		As IV	As IV
VII Coraciiformes	8	Hornbills Scarce A Roller Staphylococci diplococci, anthracoids Humming Birds exclusively gram-positives	Hornbills Coliforms dominant factor also proteus A Roller 1 or 2 isolated gram.-neg. bacilli Humming Birds none
VIII Psittaciformes	18	mixture of organisms as Order 1	Only in 2 specimens isolated coliforms, 16 specimens none
IX Strigiformes	1	few fungi	rich growth of coliforms
X Accipitriformes	1	none	*Aerobacter cloacae*
XVI Anseriformes	1	none	coliforms only
XXIV Sphenisciformes	1	none	coliforms
XXVII Ralliformes	1	Anthracoids, gram.-positive diplococci	coliforms
XXVIII Columbiformes	2	A pigeon gram-positive diplococci, yeast anthracoids	A pigeon none

NOTES AND ABSTRACTS

MEETINGS OF THE SOCIETY FOR SCIENTIFIC BUSINESS

14th October 1958

Sir LANDSBOROUGH THOMSON, C.B., O.B.E., D.Sc.

President, in the Chair.

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : JUNE, JULY, AUGUST AND SEPTEMBER, 1958

By L. HARRISON MATTHEWS, M.A., Sc.D., F.R.S.

The registered additions to the Zoological Society's Menagerie in London during the months of June, July, August and September were 499 in number, including some domestic animals. The following are of special interest : a Giant Panda, *Ailuropoda melanoleuca*, originally deposited, later purchased ; a very fine collection of twenty-five Humming Birds, presented by H.E. The Brazilian Ambassador, including the following which are new to the collection : an Amethyst Humming Bird, *Calliphlox amethystinus* (Boddaert), ten Brazilian Wood Nymph Humming Birds, *Thalurania glaucopis* (Gmelin) and five Brazilian Ruby Humming Birds, *Clytolaema rubricauda* (Boddaert) ; a Kenya Vitelline Weaver Bird, *Ploceus vitellinus uluensis* (Neumann) presented by Dr Edwin Lindsey ; a Southern Painted Terrapin, *Chrysemys picta dorsalis* Agassiz presented by Mrs M. Bennett Towbis ; two Iberian Grass Snakes, *Natrix natrix astreptophora* Seoane, presented by Mr Peter Hopkins; a Himalayan Ruby-Throat, *Erithacus pectoralis pectoralis* (Gould), received in exchange. All new to the collection. Two Malayan Palm Civets, *Paradoxurus hermaphroditus musanga* (Raffles) presented by Mr A. W. Cookson. This subspecies has not been recorded previously, but as specimens have been received from Malaya in the past, they have probably been exhibited as *P. hermaphroditus*. Two Yellow-throated Martens, *Martes flavigula*, male and female, presented by Dr K. C. Searle, C.M.Z.S. ; a Humboldt's Woolly Monkey, *Lagothrix humboldti*, presented by Mrs E. M. Payne ; a White-throated Capuchin Monkey, *Cebus capucinus*, presented by Mr A. M. Braham ; a Frilled Lizard, *Chlamydosaurus kingi*, purchased ; an Alpaca, *Lama pacos* ; a Mandrill, *Mandrillus sphinx* ; a Giraffe, *Giraffa camelopardalis* ; and a Short-tailed Wallaby, *Macropus brachyurus* have been born, and a Sulphur-crested Cockatoo, *Kalatoe galerita* ; a Guiana Parrotlet, *Forpus passerinus passerinus*, and three Gough Island Moorhens, *Porphyriornis comeri* have been hatched in the Menagerie. Two Wolverines, *Gulo gulo*, received in exchange. During the same period there

were 231 additions to the Menagerie at Whipsnade Park, apart from transfers from London.

The following communications were read and discussed :—

Dr W. C. Osman Hill. Aborted mid-term foetus of *Aotes trivirgatus infulatus* (Feline Douroucouli).

On the 30th July 1958 the pregnant female of a pair of Three-banded Douroucoulis (*Aotes trivirgatus*) received two weeks previously, miscarried. The foetus and membranes were obtained complete and proved to be of considerable interest. The bidiscoidal placenta comprises a main disc, 31·5 × 23·5 mm., of approximately triangular outline with rounded angles, to which the long funis (105 mm.) is attached somewhat excentrically, and a more rounded subsidiary disc, 27·5 × 25·8 mm. The subsidiary disc nowhere touches the main disc, but receives its vessels marginally after these have crossed the smooth chorion from the main disc.

The foetus, 87·5 mm. CR length, is chiefly of interest in view of the extraordinarily un-monkeylike appearance of the head which, both in proportions and shape, recalls that of a foetal tarsier (*Tarsius bancanus borneanus*) of approximately equivalent development described by Wood Jones (*Proc. zool. Soc. Lond.* 1951, p. 723). Thus the head, especially the muzzle, is elongated, and the eyes are very large, though to a less extreme than in the Tarsier. The relative proportions of the cranial parts of the two foetus, compared with the respective adults are indicated in the subjoined table :—

PROPORTION OF HEAD STRUCTURES TO TOTAL HEAD-LENGTH (rhinarium–occiput)

	Aotes foetus per cent	*Aotes* adult per cent	*Tarsius* foetus per cent	*Tarsius* adult per cent
Orbital diameter	25·5	28	30	50
Palpebral fissure	20	20·5	26	40
Ear length	30	44	30	69
Ear breadth	24	32·3	30	52

These data reopen the long-disputed question as to the affinities of *Aotes*. Current opinion is that any likenesses between the nocturnal *Aotes* and the prosimian Primates is due purely to convergence, by adaptation to similar mode of life, but several earlier workers (notably Humboldt, 1811, p. 306; Wagner, 1837 ; Ménégaux, 1902 and Ribeiro, 1940) have postulated a genetic connection between the lemurs or *Tarsius* and *Aotes*. The presence of tarsioid appearances in the advanced foetus of *Aotes* shows the need for further investigations of other developmental stages in this genus and in that of its supposed relative *Callicebus* which is, in fact, more generalised than *Aotes*.

Mr R. N. T–W–Fiennes. Report of the Pathologist for the year 1957. [Published in the ' Proceedings ' Volume 132, Part 1].

Dr Desmond Morris. The behaviour of the chimpanzee in captivity. (Illustrated by a film).

11th November 1958

Sir Landsborough Thomson, C.B., O.B.E., D.Sc.

President, in the Chair.

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : OCTOBER 1958

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions to the Zoological Society's Menagerie in London during the month of October were 122 in number, including some domestic animals. The following are of special interest : two Chinese Leopards, *Panthera pardus japonensis* (Gray), new to the collection ; a Maned Wolf, *Chrysocyon brachyurus*, purchased ; a Snow Leopard, *Panthera uncia*, received in exchange ; a Brown Hyaena, *Hyaena brunnea*, purchased ; a Uganda Green Pigeon, *Treron australis granyiki* Grote, new to the collection, presented by Mr J. R. Newmark ; and a Mantell's Kiwi, *Apteryx australis mantelli*, presented by the Government of New Zealand. During the same period there were nine additions to the Menagerie at Whipsnade Park, apart from transfers from London.

The following communications were read and discussed :—

Professor M. Millott and Mr M. Yoshida. The photosensitivity of the sea-urchin *Diadema antillarum* : responses to increases in light sensitivity. [To be published in the ' Proceedings ' Volume 132].

Dr Desmond Morris. The analysis of the shell-smashing behaviour of certain vertebrates. (Illustrated by a film).

Mr P. L. Wayre. The Gyr-Falcon in Iceland. (Illustrated by a film).

9th December 1958

N. D. Riley, Esq., C.B.E.

Vice-President, in the Chair

REPORT ON THE ADDITIONS TO THE SOCIETY'S MENAGERIE : NOVEMBER 1958

By L. Harrison Matthews, M.A., Sc.D., F.R.S.

The registered additions to the Zoological Society's Menagerie in London during the month of November were 130 in number. The following are of special interest :—two Cheetahs, deposited by H.R.H. The Duke of Gloucester ; three Libyan Striped Weasels, *Poecilictis libyca*, presented by Dr Harry Hoogstraal. These are believed to belong to a subspecies which has not yet

been named. Two Arabian Spiny Mice, *Acomys cahirinus dimidiatus* Cretzschmar, new to the collection, received in exchange ; two Marco Polo's Sheep, *Ovis ammon polii* Blyth, new to the collection and two Saiga Antelopes, *Saiga tatarica*, received in exchange from the Zoo Centre, Moscow ; a Formosan Black Bulbul, *Microscelis nigerrimus* (Gould), new to the collection, presented by Mr J. R. Newmark ; and sixteen Bordered Sedge Frogs, *Hyperolius marginatus* Peters, new to the collection, presented by Dr W. M. Fitzsimmons. During the same period there were twenty-seven additions to the Menagerie at Whipsnade Park, apart from transfers from London.

The following communications were read and discussed :—

Dr F. C. Fraser. Exhibition of a film, taken by Mr William E. Schevill of Woods Hole, Massachusetts, at Silver Springs, Florida, of the Amazonian River Dolphin, *Inia geoffrensis*.

Dr E. H. Ashton and Mr C. E. Oxnard. Variation in the maxillary nerve of certain mammals. [Published in the ' Proceedings ' Volume 131 Part 4].

Dr Patricia Scott, Mr. J. P. Greaves and Mr M. G. Scott. Calcium and iodine deficiencies in cats fed on a meat diet. (Illustrated by a film).

Mr John Hillaby. The fauna of Ruwenzori, the Mountains of the Moon (Illustrated by a coloured film).

NOTICE

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References in the text must be cited by giving the author and date, e.g. " Smith (1957) stated . . . " and the complete references arranged in alphabetical order of the authors' names at the **end of the paper.** They must contain the following information, in the order given : (1) The author's name(s) and initials(s). If there is more than one author the ampersand (&) not the word " and " must be used between the last and the penultimate names. (2) The publication date of the paper, in brackets. If reference is made to more than one paper by the same author(s) published in a single year, the suffixes a, b, etc. should be used, e.g. (1951 a). (3) The full title of the paper. Only the first word, except proper names, may have a capital letter. Titles in German have capitals as in the original journal. (4) The title of the journal abbreviated according to the " World list of scientific periodicals ". (5) The volume number in arabic numerals without the prefix " vol." and the number of the first page in arabic numerals, without the prefix " p." If the journal has appeared in more than one series the series number must appear in brackets before the volume number, e.g. (3), (n.s.) (B).

Where the reference is a separate book the title must be underlined and the place of publication given.

References thus appear as follows :—

PARRINGTON, F. R. (1956). The patterns of dermal bones in primitive vertebrates. *Proc. zool. Soc. Lond.* **127,** 389.

COWLES, R. B. (1956). Sidewinding locomotion in snakes. *Copeia* **1950,** 211.

ROMER, A. S. (1956). *Osteology of the reptiles.* Chicago.

GREEN, J. (1956). Variation in the haemoglobin content of *Daphnia*. *Proc. roy. Soc.* (B) **145,** 214.

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